PCR troubleshooting guide

PCR problems can be of the following kinds:

- Reagent problems
- Template problems
- Primer problems
- Problems with cycling conditions
- Operator errors

Accordingly, corrective actions must involve removing the source of the problem (especially if that source is the operator). Just kidding.

Reagent problems:
The simplest problem to diagnose is whether the reagents (that is the PCR buffer, the polymerase, the nucleotides, etc.,) are functional. For this, a positive control, namely a template-primer set that has been shown to work in the past, is used, under suitable conditions, to check that the reaction proceeds normally.

Note that the positive control can be any template-primer set that you know works well. You will not always be able to use the same template as in the experimental reaction simply because you may never have worked with that template before and have no other primers that you know will work with it. Because of this, your positive control may confirm that your reagents are fine, but *cannot provide you with information about your experimental template and primers, nor can it tell you whether the cycling conditions that work for the positive control are appropriate for your experimental reaction.*

Obviously, if the positive control does not amplify, then you need to start over with fresh reagents. The first suspects among the reagents will generally be either the enzyme or the dNTPs, since the buffer and magnesium chloride solutions are relatively stable. If the reagents are shown to be fine, you can then proceed to look for other sources of problems.

Template Problems:
Template problems can be tricky mainly because they can be hard to identify until other problems have been eliminated. Assuming that the template actually contains the target sequence you want to amplify (do your sequence analysis properly before launching into PCR) you can check the template (remember this could be genomic DNA or RNA depending on whether you are doing regular or RT-PCR) for concentration, purity (spectrophotometer, A260/280 ratios) and integrity (run a gel) before use in the reaction. This will tell you that you have template, and that you have sufficient template. It will not, of course, assure you that there is not something in the template that inhibits DNA polymerase, for instance.

If you have plenty of intact template, you can check for inhibitors in the template prep by adding different amounts of the template prep to a working control reaction. If there is an inhibitor present in the template prep, it should inhibit the control reaction in a concentration dependent manner.