

Ten Things That Can Kill Your PCR (by Peter Frame)

A blank PCR gel has got to be one of the most aggravating things about molecular biology. We've all had PCRs work one day, then fail inexplicably the next. And we've all banged our heads against a wall trying to figure out what went wrong. If your gels are turning up blank, check out this list of things that can kill your PCR -- and get your experiments working!

Nowhere is the old adage "an ounce of prevention is worth a pound of cure" more applicable than to PCR. Depending on your level of PCR expertise, some of these hints may seem obvious. But how many times have you kicked yourself because you overlooked the obvious? Whether you're an expert or a novice, any of these may be just what you're searching for to get your PCR working.

1. Too much dNTP, or degraded dNTP. Too much dNTP can actually inhibit your PCR reaction. Between 40 - 200 μ M is the optimal range. Also, dNTPs are sensitive to repeated freeze-thaw cycles. Make small aliquots when you get a fresh batch, and turn over your stock frequently since dNTPs frozen at -20 C will eventually go bad.
2. Not mixing $MgCl_2$. Magnesium chloride solutions form a concentration gradient when frozen and need to be vortexed prior to use (1).
3. Wrong $MgCl_2$ concentration. Every PCR reaction has an optimal $MgCl_2$ concentration range, usually between 1 - 4 mM. Mg^{2+} ions form complexes with dNTPs and can also act as a co-factor for polymerases, so you'll need to try several conditions to optimize your concentration.
4. Inhibitors in your reaction. Make sure you know how you got your source DNA. Chloroform, phenol, EDTA, ionic detergents (SDS and Sarkosyl), xylene cyanol, bromophenol blue and ethanol -- among many other things -- can inhibit PCR. An extra clean-up step on your template may do the trick. Also, certain polymerases can be more susceptible to certain substances, so be sure to check your polymerase for possible inhibitors.
5. Poor quality mineral oil. Lower-grade preparations may contain nucleases that can kill your PCR. Also, avoid autoclaving your mineral oil if possible. Exposure to high heat may cause reaction-inhibiting hydrocarbons to form. Similarly, do not irradiate mineral oil with UV for long periods (2).
6. Too much enzyme. Excess enzyme in your PCR can lead to smearing of PCR products. Most people seem to use 0.5 μ l of their stock Taq per reaction, but that may contain way more than necessary for your particular reaction.
7. Wrong primer concentration. If you have too little primer you won't see any product. Too much primer and you may get primer dimerization and not enough amplification. Stay within 0.1 - 1.0 μ M of primer.
8. Wrong PCR program. Make sure the program you selected on your PCR machine is actually the one you want! It only takes a slip of a finger, or some klutz, to alter your personal program on a common PCR machine. Check your program while it's cycling to make sure it's what you wanted.
9. Excess or insufficient template. Too much template can inhibit PCR by binding all the primers. Too little template, and amplification may not be detectable. For 25 - 30 cycles, 10^4 copies of the target sequence are sufficient.
10. Poor primer design. While primer design can seem like a black art, avoid obvious errors like self-complementarity, complementarity between paired primers, or excessively long oligos (>30 bp). Often, making a new primer next to a suspect one can solve the problem and can be faster and cheaper than trying numerous variations in reaction conditions.

References:

1. <http://www.md.ucl.ac.be/didac/sbim2520/pcr.html>
2. Dohner DE, Dehner MS, Gelb LD. Inhibition of PCR by mineral oil exposed to UV irradiation for prolonged periods. *Biotechniques* 1995; 18(6): 964-7.

Taken from: http://www.biowire.com/nucleus/nucleus_1_1.jsp