Avoid DNA contamination in PCR with these tips

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Obtaining a clean, successful PCR requires samples free of exogenous DNA. But contaminating DNA can be lurking around every corner—from previously amplified products hanging out in the lab to your own DNA. The good news is that you can largely avoid common types of contamination by following these simple guidelines:

1. Designate and use distinct areas for sample preparation, PCR setup, and post-PCR analysis. To avoid contamination from old amplicons, set up the stations on separate benchtops, one for pre-PCR (for PCR reaction setup only) and the other for post-PCR (purifying PCR-amplified DNA, measuring DNA concentration, running agarose gels, and analyzing PCR products).
2. Restrict equipment to these areas. Keep the PCR machine and electrophoresis apparatus in the post-PCR area.
3. Prepare and store reagents for PCR separately and use them solely for their designated purpose. Aliquot reagents in small portions and store them in either location based on their use in pre-PCR or post-PCR applications. Store the aliquots separately from other DNA samples.
4. Use separate sets of pipettes and pipette tips, lab coats, glove boxes, and waste baskets for the pre-PCR and post-PCR areas. If you're doing NGS library prep, use a surface decontaminant for nucleic acids to wipe down benchtops and pipettes before you begin.
5. Use pipettes and pipette tips with aerosol filters dedicated for DNA sample and reaction mixture preparation.
6. Follow the golden rule of PCR: **DO NOT bring any reagents, equipment, or pipettes used in a post-PCR area back to the pre-PCR area.** This even goes for your lab notebook and pens. Label pre- and post-PCR items, so it's easy to tell where they belong.
7. Keep the number of PCR cycles to a minimum, as highly sensitive assays are more prone to the effects of contamination.

Identify Your Contamination Source

Where this contamination could be coming from? Well, anywhere really. It could be coming from **1) your laboratory environment** such as your pipettes, tips, hands, bench top, centrifuge, and so on, or **2) your reagents** such as your polymerase, buffer, nucleotides, water, and other reagents.

1) Rule out Your Laboratory Environment

First thing first, remove all possible environmental sources, when tracking down PCR contamination. To do this you should:
Use a 10% bleach solution or DNA-away to wipe down your:

- Bench top
- Pipettes
- Centrifuge
- Vortex
- Racks
- Thermocycler lid and buttons

Get new:

- Unopened filter tip boxes
- Unopened sterile PCR tubes

Correctly assemble your PCR reaction:

- **Wear a dedicated lab coat.** You should wear a lab coat dedicated to PCR setup. This should NOT (!) be the same coat you wear when analyzing your PCR results. This coat should not go anywhere near open tubes of amplified PCR product.
- **Change your gloves frequently.** If you leave your PCR setup and dedicated equipment to do anything—get more reagents, answer your phone, use a pen—change your gloves before returning to the setup.
- **Use only dedicated equipment.** The pipettes, centrifuge, and vortex you use when setting up a PCR should be dedicated to PCR setup. No amplified PCR products should come anywhere near them.

2) Rule out Your Reagents

Now that you have minimized the introduction of any new PCR contamination into your PCR assembly, it is time to check your reagents. This means systematically substituted each of your old reagents with a new (previously unopened) reagent and re-running your negative control. Whatever substitution(s) removes your contamination bands is the contaminated reagent, which should be discarded.

Avoid Future Contamination

To avoid future contamination, you should do everything above and a few more things:

- **Work in dedicated space.** Setup your PCR away from where you analyze PCR results. This is best done in a hood or, at minimum, benches away from where you run gels.

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- **Store PCR reagents and PCR products separately.** In the same way that you do not want your PCR analysis to be near where you setup, you should never store PCR product and reagents together. Whenever possible use separate refrigerators.
- **Aliquot.** No matter how careful you are, contamination can still happen. Contamination costs not just time but also money, as you must throw out any contaminated reagents. If that reagent is new, then that can mean $300 straight into the trashcan! So, whenever possible, aliquot your reagents into smaller tubes and only work from one aliquot at a time. Not only will this help you deal with contamination, but it also prolongs the life of your reagents by reducing the number of freeze/thaw cycles.
- **Store PCR tubes/tips/racks separately.** Keep your PCR stuff clean by storing it well away from PCR product. Label all PCR equipment as such and tell other lab members to respect their designation.
- **Don't flick your tubes open.** Minimize aerosolizing your PCR product by never "flicking" your PCR tubes open. I know it is faster to open your tubes with one hand by flicking the lid open with your thumb, but this is bad form and contributes to your bench's contamination. Instead take your time and use two hands to carefully open all PCR product tubes.
- **Use a master mixer and add your template last.** The less times you handle your template, the less opportunity there is for contamination. Therefore, you should always set up your PCRs using master mixes made with (in order): water, buffer, nucleotides, primers, polymerase, and, finally, template.
- **Train others.** The cleanliness in your lab is only as strong as your weakest lab member. Inform others about PCR contamination, and the steps needed to avoid it. Hey, you could even print this article and share it with them.

Good luck and happy PCR-ing!

Thanks!

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