

CONTAMINATION

Identifying and Preventing DNA Contamination in a DNA-Typing Laboratory

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INTRODUCTION

One of the biggest strengths of PCR^(e) for DNA typing is the degree to which DNA can be amplified. Starting with a single DNA molecule, millions or billions of DNA molecules can be synthesized after 32 cycles of amplification. This level of sensitivity allows scientists to extract and amplify DNA from minute or degraded samples and obtain useful DNA profiles. In this context, the sensitive nature of PCR works in a lab's favor, but it can cause problems if great care is not taken to avoid contaminating the reaction with exogenous DNA. Three main categories of exogenous DNA have the biggest impact on DNA-typing laboratories. These are: 1) DNA from the analyst, 2) DNA from other samples in the lab, and 3) DNA fragments of the allelic ladder used to determine the size of amplified alleles. DNA from nonhuman sources, such as bacteria contaminating a casework sample, will not be amplified and detected because STR systems are species-specific (1). Here we discuss ways to detect DNA contamination and present tips to minimize contamination problems.

In a DNA-typing laboratory, every precaution must be taken to ensure that results are accurate and unexpected peaks or bands do not cast doubt on the data.

DETECTING DNA CONTAMINATION

Many labs determine the DNA profile of their analysts, so contamination with an analyst's DNA can be detected by the appearance of his or her profile in the negative control reaction. Contamination of a reaction with allelic ladder usually results in a clear pattern that includes a number of alleles present in the allelic ladder for all loci analyzed. Typically smaller loci are preferentially amplified, but if the level of contamination is high enough, many alleles in most loci will be amplified to detectable levels. Fortunately, this source of contamination is often easy to identify. Detecting contamination with DNA from other samples can be much more problematic. It is often difficult to distinguish low-level contamination with another DNA sample from a true mixture of DNA templates. For this reason, laboratories have strict protocols and safeguards to prevent DNA contamination, and most laboratories perform proficiency tests to ensure that contamination is a rare event. Proficiency tests are often laboratory-specific and, therefore, will not be discussed here.

Appropriate control reactions are helpful in determining whether DNA contamination has occurred. A "reagent blank" control consists of all reagents used during sample processing but contains no sample. This control is used to detect DNA contamination of the analytical reagents used to prepare the sample for analysis. In a separate negative control reaction, water is used instead of extracted sample or reagent blank. This negative control reaction is often referred to as the "no-template" control and allows identification of contamination in the amplification reagents themselves. The reagent blank and no-template controls are effective in detecting contamination because there is no added DNA template to compete with minute amounts of contaminating DNA for amplification. However, contamination can also be detected based on the results from a positive control template. The presence of unexpected peaks in the negative or positive control reactions can indicate DNA contamination. For illustrative purposes, we will focus on allelic ladder contamination in the following discussion.

IDENTIFYING ALLELIC LADDER CONTAMINATION

Allelic ladders contain a high concentration of DNA fragments. Therefore, the smallest trace of allelic ladder can be amplified to detectable levels during PCR. In the simplest cases, allelic ladder contamination results in a clear pattern that includes some or all alleles present in the ladder (Figure 1). These patterns can be easily misinterpreted as the presence of “extra” stutter peaks, also known as “n-4” peaks for tetranucleotide loci, because peaks appear at the n-4, n-8, n-12, etc., positions (or n-5, n-10 and n-15 for pentanucleotide loci).

However, the detection of allelic ladder contamination is not always this simple. The contaminating allelic ladder may be from a different STR system. If the primer sequences are identical or the primers are nested, additional peaks corresponding to alleles of loci common to both systems appear. For example, if the GammaSTR® Multiplex (Fluorescein) D16S539, D7S820, D13S317, D5S818 Allelic Ladder Mix^(b) is introduced into a negative control reaction assembled using the PowerPlex® 16 System, many of the alleles present in the GammaSTR® Multiplex Ladder Mix will be detected (Figure 2).

OTHER POSSIBLE EXPLANATIONS FOR UNEXPECTED PEAKS

When checking for additional peaks, be aware of other possible sources of unexpected peaks that are not indicative of DNA contamination. For example, stutter peaks or n-1 peak heights will be high if excessive amounts of DNA template are used or samples are overloaded. To remedy this, use less DNA template (we recommend 0.5–1.0ng of template per reaction) or reduce the number of

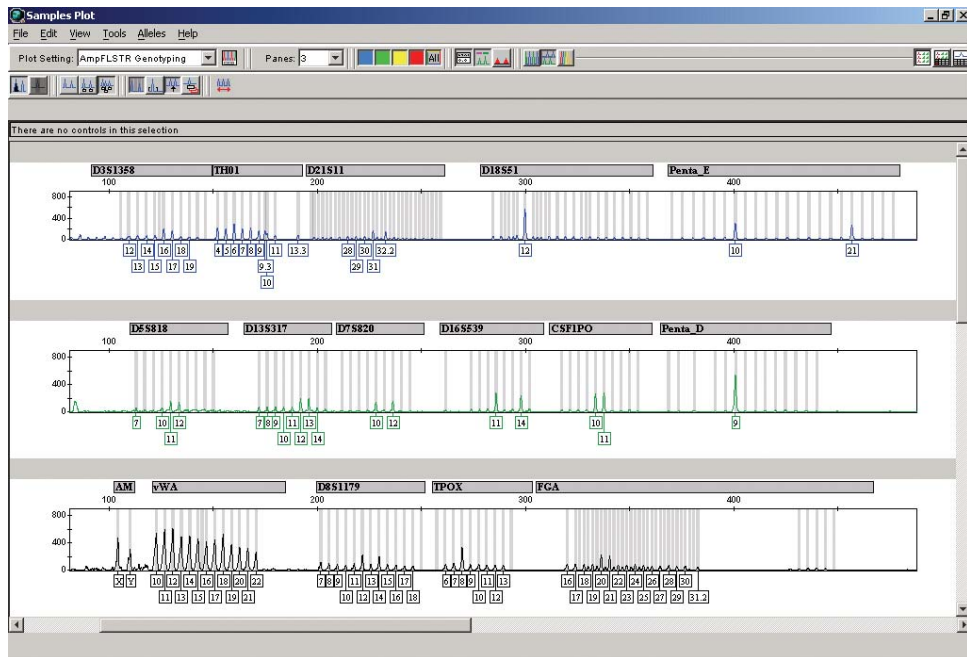


Figure 1. An example of allelic ladder contamination in a PowerPlex® 16 sample amplification. Sample peaks are evident but are masked by the amplified allelic ladder fragments, especially in the smaller loci.

cycles in the amplification program by 2–4 cycles (10/20 or 10/18 cycling). Alternatively, reduce the injection time for capillary electrophoresis (CE) instruments or the amount of amplification product loaded onto the gel. Also, be sure that the samples are completely denatured by heating the samples for the recommended time and cooling on crushed ice immediately prior to loading the gel or capillary. CE-related artifacts (“spikes”), such as those caused by minor voltage changes or urea crystals passing by the laser, may also result in unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Contaminants in the water used with the ABI PRISM® 310 Genetic Analyzer or other CE instruments may generate unexpected peaks in the blue and green dye colors. This includes the water used to dilute the 10X Genetic Analyzer

buffer. Use autoclaved water, change vials and wash the buffer reservoir as a part of normal weekly maintenance of the instrument.

Unexpected peaks can also be caused by pull-up or bleedthrough. Pull-up can occur when a poor or incorrect matrix has been applied to the samples. If the peak heights are excessive, re-inject samples using a shorter injection time or load less sample on the gel. If the matrix or spectral calibration is not performing optimally, generate a new matrix and apply it to the samples (ABI PRISM® 310 and 377 instruments), or perform a new calibration and re-analyze the samples (ABI PRISM® 3100 and 3130 instruments).

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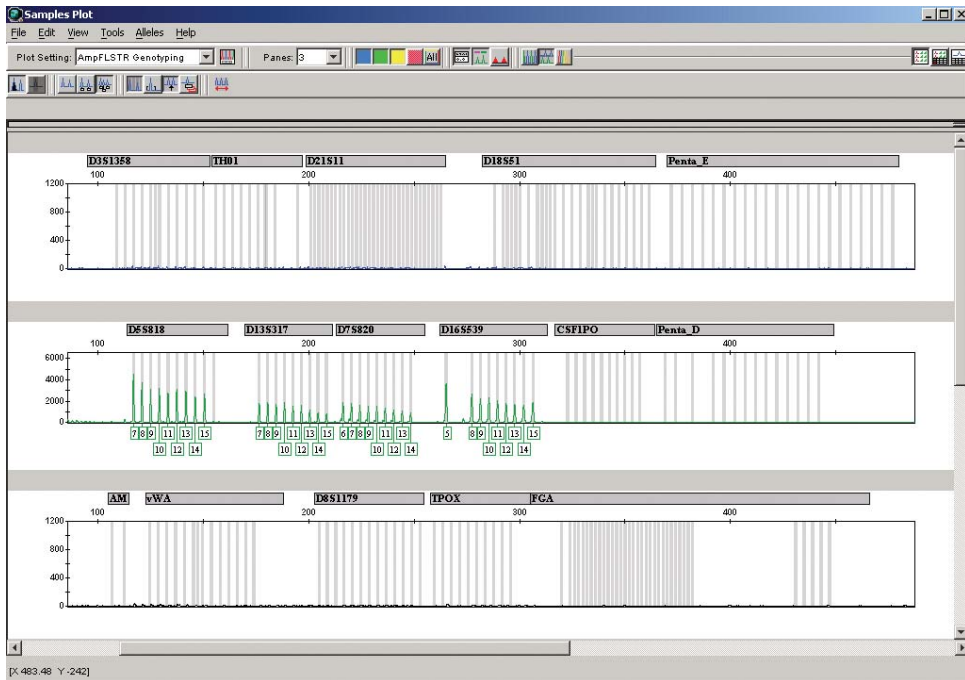


Figure 2. Contamination of a no-template negative control PowerPlex® 16 reaction with allelic ladder from the GammaSTR® Multiplex (Fluorescein) D16S539, D7S820, D13S317, D5S818.

RESOLUTION

If DNA contamination occurs, there are steps that can be taken to eliminate the contaminant. Individual solutions can be identified as contaminated and discarded. To check solutions for contamination, assemble negative control reactions using new reagents known not to be contaminated, and add one of the suspect solutions to each reaction. A reaction that shows amplified products indicative of contamination is evidence that the solution added was contaminated.

However, it is often easier, less time-consuming and more reassuring to simply discard all solutions that have come into contact with samples that demonstrate contamination.

Wash all surfaces and rinse pipette barrels with a dilute bleach solution (2% to 3%). Be sure to rinse any equipment that comes into contact with reagents well, since residual bleach can cause allele or locus

dropout. For equipment that cannot be treated with bleach, exposure to ultraviolet light can eliminate DNA contaminants (2–4).

Once the laboratory equipment has been cleaned and any questionable reagents discarded, assemble a number of reactions without DNA template. Carefully examine the amplification results looking for additional peaks that can indicate continued DNA contamination problems.

PREVENTING CONTAMINATION

One of the most efficient safeguards to prevent contamination is the separation of pre- and postamplification areas and equipment. Use a dedicated set of pipettes, preferably with aerosol-resistant (barrier) tips, in the pre-amplification area. Do not enter the pre-amplification area after handling amplified samples or allelic ladder. Many analysts plan their day so that

pre-amplification work is performed first, and postamplification work is performed later in the day. Do not perform DNA purification in the pre-amplification area. In the absence of a separate pre-amplification area, reactions can be assembled in a sterile hood that is reserved solely for this purpose.

The use of gloves is essential; be sure to change gloves whenever you move from the pre-amplification area to postamplification areas. As much as possible, avoid touching any surfaces or objects with your gloved hands. For every set of reactions, assemble a negative control reaction, and scrutinize the reactions for the presence of unexpected peaks. After setting up reactions, wash all surfaces with a dilute bleach solution.

CONCLUSION

In a DNA-typing laboratory, every precaution must be taken to ensure that results are accurate and unexpected peaks or bands do not cast doubt on the data. In this article, we showed examples of reactions contaminated with allelic ladder, discussed methods to eliminate DNA contaminants and offered tips to prevent DNA contamination in the future. By diligently following these recommendations, you can prevent DNA contamination from ever becoming a problem in your laboratory.

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