

Control of Contamination Associated with PCR and Other Amplification Reactions

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INTRODUCTION TO CONTAMINATION CONTROL

A molecular diagnostic laboratory that plans on using one or more in-vitro amplification reaction(s) (IVAR) should also be evaluating measures to control a contamination problem which parallels the use of these procedures. Historically, the concept of contamination in a biomedical laboratory has been associated with the unintentional disbursement of radioisotopes (e.g., ^{32}P , ^{35}S , etc.) in areas not designated for their use. Removal of these radioactive species was typically straightforward, rapid, and effective. Indeed, trace amounts of these radioactive species have usually been removed without problem and the laboratory restored to its original non-contaminated condition.

However, the molecular genetics laboratory that has become contaminated with a biological species faces a much more difficult problem. Since the advent of the polymerase chain reaction (PCR)*, the molecular genetics laboratory has possessed an experimental capability of enormous sensitivity. Unfortunately, the PCR's exquisite capacity for amplification was accompanied by its extreme sensitivity to the presence of its own product as a feedback contaminant. [1-3] Because of the need for some molecular diagnostic laboratories to routinely detect less than 100 copies of certain target templates (e.g., viruses), this susceptibility of the PCR to trace amounts of its own product means the laboratory has a continual requirement to control a species it cannot easily detect, see or readily remove.

Although the two types of contamination (e.g., radioactivity and amplified nucleic acid) can both be considered as being unintentional, any further comparison disappears almost immediately on at least three accounts:

- A. **Longevity:** The most common radioisotope used in molecular biology has a short half-life (e.g., TH for ^{32}P = 14.3 days). As such, by waiting a certain period of time following the contamination event ($6 \times \text{TH}$), the major amount (> 98%) of the radioactive contaminant spontaneously degrades. By contrast, the main product (and corresponding contaminant) produced by the PCR (e.g., dsDNA) has been shown to exist for thousands of years.⁴ However, since some of the IVAR produce RNA instead of DNA, their products (as contaminants) will exist for much less time due to the inherent instability of RNA. Thus, IVAR that produces RNA may not present as much of a serious problem. Unfortunately, contamination produced by an IVAR that yields dsDNA can be considered permanent. Laboratories have physically moved their facilities or stopped performing a PCR assay for particular targets (e.g., viral) because the labs were permanently contaminated and negative controls (water blanks) included in the assays were consistently positive.
- B. **Detection:** Radioactive nucleotides typically used in molecular biology (e.g., ^{32}P , ^{35}S , etc.) emit beta-particles that induce a response from a Geiger counter. By contrast, nucleic acid molecules, which are produced by an IVAR and then accidentally disbursed, are virtually impossible to detect by direct means. (Note: The exception to this is when the PCR [or

other IVAR] utilizes a radioactive precursor such as one of the dNTPs or a radiolabeled amplicon to produce a radioactive amplicon.) **Amplification:** Radioisotopes do not have the intrinsic property of reproduction. Rather, the amount of radioactivity in a labeled species always begins at a fixed amount and is decreased until the last of the species has undergone the corresponding decay reaction. IVAR products, by contrast, can be reproduced to orders of magnitude (10^4 to 10^9) beyond their original concentrations. Moreover, the amount of DNA product present in even minuscule amounts (e.g., nanoliter, or less) may be sufficient to serve as a template for subsequent IVAR (e.g., PCR, LCR, RCR, etc.). (Table 1)

Table 1
In Vitro Amplification Reactions

Name	Abbreviation	Amplified Product	Enzyme(s) Needed	Reactants Needed
1. Polymerase Chain Reaction	PCR	DNA	tsDNA polymerase	4 dNTPs + 2 oligonucleotides
2. Ligase Chain Reaction	LCP	DNA	tsDNA ligase	ATP + 4 oligonucleotides
3. Repair Chain Reaction	RCR	DNA	tsDNA polymerase tsDNA ligase	(dATP and dCTP) or (dTTP and dGTP) + 4 oligonucleotides
4. Self-Sustained Sequence Replication	3SR	RNA	Reverse transcriptase RNA polymerase	4 dNTPs + 4RTPs + 2 oligonucleotides

Note: ts = thermally stable

dNTPs = dATP, dCTP, dGTP, dTTP

RTPs = ATP, CTP, GTP, UTP For example, consider a first round PCR (volume = 100 μ l) that started with 100,000 copies of a single copy gene contained in 1 μ g of genomic DNA. When this template DNA is subsequently amplified by a factor of 10^5 (nominal for the PCR), every nanoliter would contain 100,000 copies of amplicon. If a single 20 micron diameter aerosol droplet (volume = four picoliters) from this first round PCR tube is inadvertently introduced into the tube for the next PCR, there are sufficient numbers of DNA amplicons (e.g., ~400) to

serve as templates for the next PCR and thereby yield a detectable reaction product in that tube. Obviously, controlling aerosols containing droplets of less than 50 microns in size is critical for preventing this type of contamination. This is especially true if a high number of thermal cycles is used for the PCR.

METHODS TO CONTROL CONTAMINATION

At present, there are a number of generally recognized procedures that have been reported with regard to controlling IVAR contamination.[5-9] These procedures will be discussed later in greater detail. The following section will focus on more recent methods that have been specifically developed to minimize or eliminate contamination due to amplicon carryover. These newer methods can be grouped in two broad categories: pre- and post-amplification treatments of the reaction tube contents. It should be mentioned, however, that no effective method is known which can remove an IVAR amplicon from a contaminated laboratory. In this case, the choices are limited to abandoning either the assay for that particular template or the laboratory where the template is currently assayed. This very unattractive choice provides strong incentives for a laboratory to deal with this problem on a prospective basis.

DETECTION OF CONTAMINATION

Before discussing contamination control procedures, a brief mention should be made about how IVAR amplicon contamination can be detected. In this context, contamination is defined as the unwanted presence of DNA (or RNA) amplicons that may serve as a future IVAR template. At present, no experimental means exist which can directly detect the ultra-trace amounts of this template (e.g., picogram amounts or less). The best way to monitor for IVAR template contamination BEFORE it becomes a serious problem is to use reagent-only ("template-negative") blanks which are prepared and included with the other samples in each IVAR run. [1,5,6] These controls contain all of the required reaction constituents EXCEPT the template (sterile distilled water can be substituted for the template). The presence of contamination is then signalled by the appearance of the test amplicon in the reagent-only blank tubes.

1. **Reagent-Only Controls:** These should also be used to check for contamination in newly prepared batches of IVAR reagents (e.g., 10X buffer, dNTPs, amplimers, etc.) before they are aliquotted or used for routine analysis. To monitor for this contamination, the new reagent is substituted for H₂O in the reagent-only blank. Once analyzed in this manner and found to be free of contamination (as evidenced by the absence of IVAR amplicons), the new reagent should be dispensed into small volume aliquots and frozen. Sufficient aliquots should be thawed for preparation of one mastermix, and then discarded. Under no circumstances should individual reagent aliquots be re-used.

CONCEPTS OF CONTAMINATION CONTROL

As indicated earlier, there are two major approaches to controlling IVAR contamination. Generally, these philosophies can be applied to any of the in vitro amplification techniques. These control techniques focus primarily on activities performed before or after the IVAR has occurred. Several may actually be found under both headings as illustrated below. Considering the intense amount of interest in IVAR contamination and the profound implications of its impact on diagnostic and research applications, alternative philosophies can be expected to emerge in the future as well.

A. Pre-Amplification Contamination Control

This approach concentrates on activities that precede the actual amplification reaction. Several different methods can be found under this heading. One involves controlling or eliminating aerosols in handling and delivering liquids used in preparing reagents for the reaction.[5,6,8] Two experimental approaches have evolved for accomplishing this: 1) the use of positive displacement tips for pipettors, and 2) the use of aerosol resistant tips. Since there are numerous pipetting steps involved in setting up an IVAR, elimination of aerosols that may contain 100 to 1000 copies of the amplifiable template is paramount. A detailed examination of each device is given below.

1. **Positive Displacement Pipettors:** When first introduced, only one brand of pipettor used positive displacement tips (i.e., disposable plunger and barrel combination). Although these tip sets came in several sizes (e.g., 20 μ l, 200 μ l, etc.), they had to be manually assembled into functional units then autoclaved before use. With the advent of PCR, the demand for these pipet tips grew very rapidly and the imported tips were, unfortunately, rationed for a brief period of time. Since then, at least two other domestic pipet manufacturers have started to produce positive displacement tips which are also of two-piece construction (plunger and barrel). One of these newer brands comes preassembled and can be directly autoclaved. The use of these tips prevents the carryover which results in contamination (Figure 1). The cost for the assemble units are \$0.35-0.37 each.

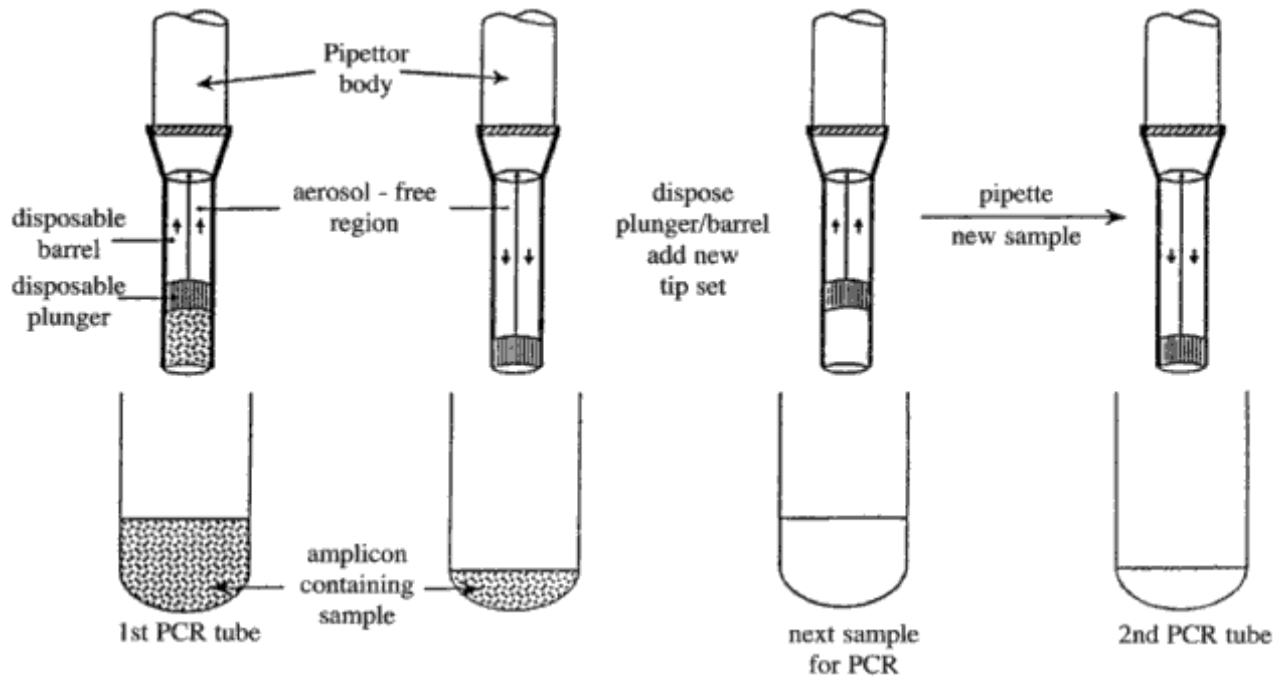
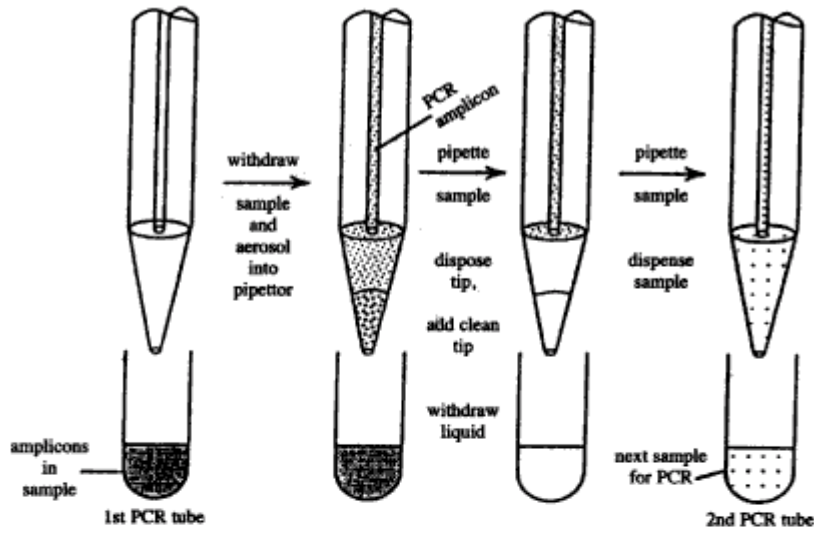


Figure 1: Use of a positive displacement tip illustrates that no aerosols are carried into the tube for the 2nd round of PCR.

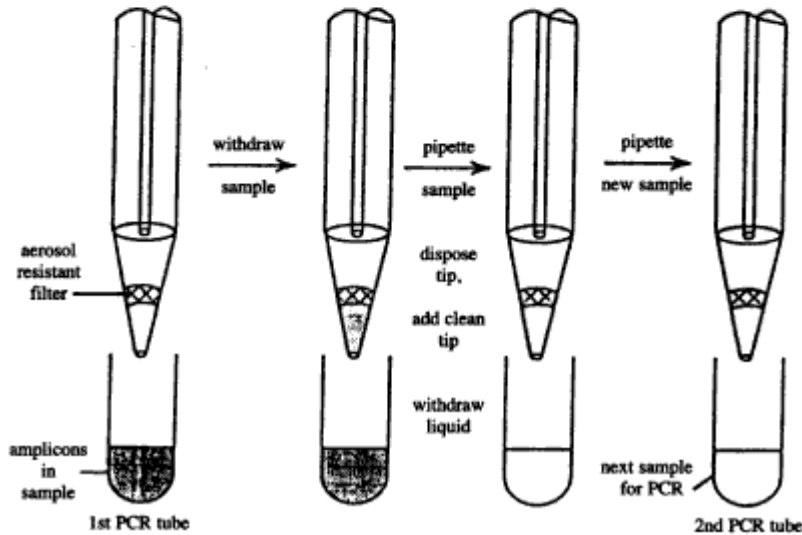
2. ART Self-Sealing-Barrier Tips: As an alternative to the positive displacement tips, Molecular Bio-Products, inc. (San Diego, California), developed the Aerosol Resistant Tip (ART). These specialized tips are now available in over 20 sizes, ranging from 10 μ l to 5000 μ l. ART tips fit most of the commercially available air-piston pipettors and some robotic pipetting stations. The principal feature of the ART pipet tip is a porous, self-sealing, physical barrier which is located between the pipet tip's upper section and the tip's orifice. When the plunger on the pipettor is released, air flows up through the porous barrier, leaving any aerosols trapped inside the barrier (Figure 2). It is the amplicons in the aerosol which, if deposited inside the pipettor's mandrel, could contaminate the next sample during subsequent pipetting steps. To further reduce pipettor contamination, one manufacturer has introduced a pipettor which can itself be autoclaved.

Several differences exist between positive displacement, standard and ART tips. Positive displacement tips require a special pipettor body. Although autoclavable and effective, they are expensive. Standard tips, albeit inexpensive, are not appropriate for IVAR due to problems with contamination. ART tips cannot be autoclaved due to the patented barrier, and are, therefore, pre-sterilized by electron beam radiation.

Use of Standard Pipet Tips



Use of ART Self-Sealing Barrier Tips



3.

Figure 2: Use of ART Self-Sealing Barrier Tips illustrates that aerosols generated during initial pipetting steps are restricted to lower portion of the tip.

Table 2**Comparison of Pipet Tips**

Type of Pipet Tip	Use standard micro-pipettor	Can be autoclaved	Prevents contamination
1. ART Self Sealing Barrier Tips	Yes	No	Yes
2. Positive Displacement	No	Yes	Yes
3. Standard	Yes	Yes	No

ART tips are lot certified RNase-, DNase- and Pyrogen-free, proven to prevent contamination, and cost approximately G that of positive displacement tips (Table 2). +Note: My laboratory has used ART brand tips from Molecular Bio-Products, inc., for over a year to manipulate samples and set up amplification reactions. We find them to work extremely well.

- Uracil-N-Glycosylase (UNG): Another pre-IVAR approach used to control contamination is an enzymatically-based method to treat samples, which are to be amplified by an IVAR. The method exploits the susceptibility of the deoxynucleotide uracil to the enzyme, uracil-N-glycosylase (UNG).[10] The contamination protocol is initiated by replacing dTTP, normally contained in the PCR mixture, with dUTP (Figure 3). This replacement has a minimal effect on the PCR since uracil can form complimentary hydrogen bonding to adenine almost as well as thymine. The dUTP is therefore readily incorporated into the amplicon produced by the subsequent PCR. Following completion of the first PCR, the reaction mixture with the dUTP containing amplicons can be analyzed normally. The next set of samples to be amplified using the PCR is now briefly treated with the enzyme UNG. The samples are then heated to destroy the UNG activity, and the heat-treated samples added to the PCR master mixture for the next round of PCR. During the incubation with UNG, any dUTP-amplicon that was unintentionally carried into the samples destined for the next round of amplification is subjected to enzymatic hydrolysis by UNG. The enzyme recognizes the uracil bases in both strands and cleaves the N-glycosylase bond that holds the uracil onto the deoxyribose-phosphate backbone. Cleavage (and subsequent loss) of the uracils then destabilizes the duplex strand(Figure 3).

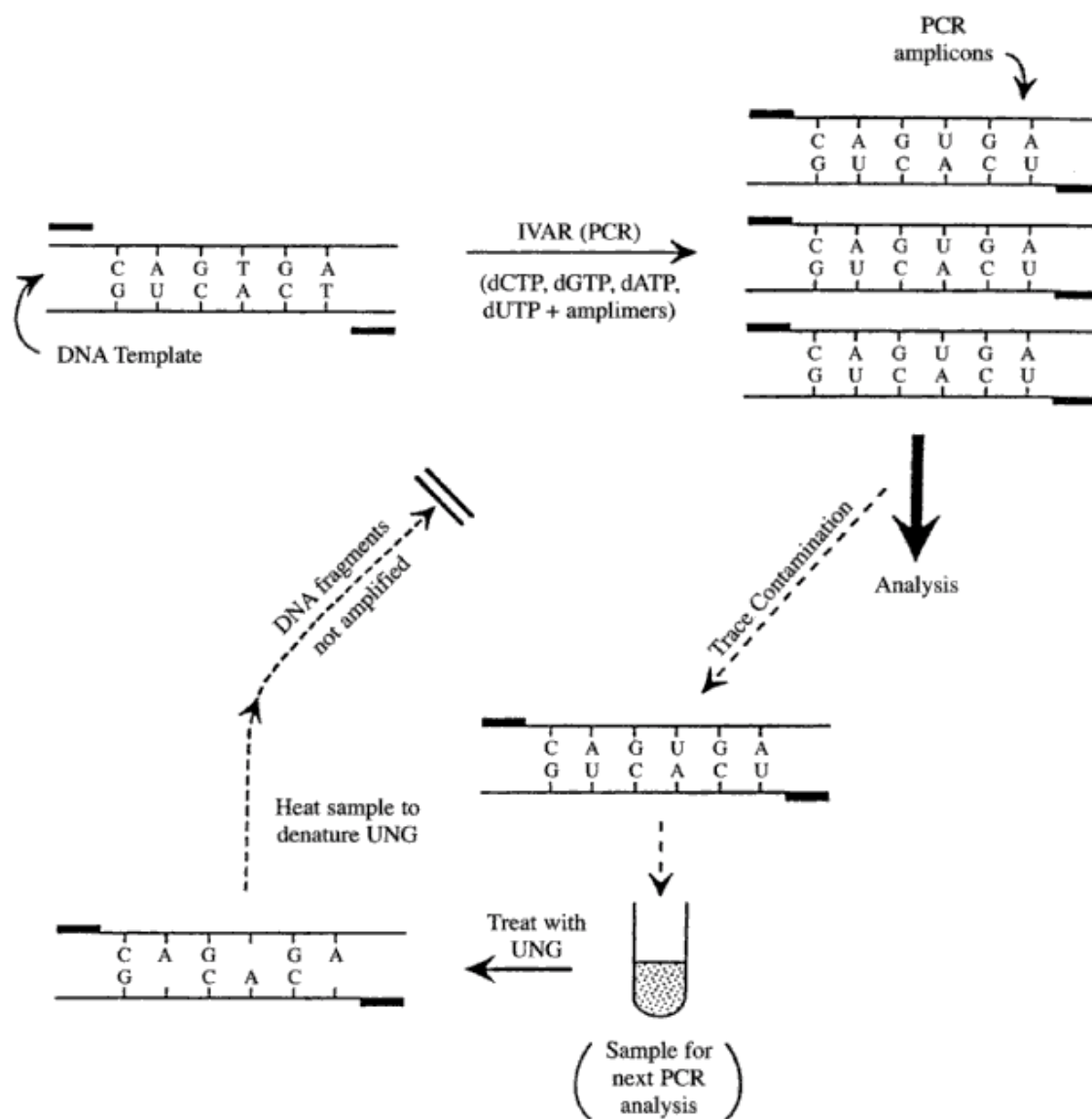


Figure 3. IVAR contamination control procedure which utilizes the enzyme uracil-N-glycosylase, UNG. The PCR amplimers bind at the sites indicated.

Several caveats are worth mentioning. First, the UNG-treated DNA is unstable to the subsequent denaturing temperatures of the next round of PCR and is degraded into smaller fragments, which are unsuitable as templates for amplification. While the replacement of dTTP with dUTP is simple, some adjustments to the PCR parameters are required and the yield of the PCR may also diminish.⁸ Second, the possibility that UNG may survive the heat denaturing step and degrade the product dUTP containing amplicon can occur.[11] Thornton et al found that if PCR

products were stored at either 4: C or 25: C following the end of the PCR, there was degradation (and loss) of amplicon product. Apparently, the UNG renatures and then degrades the dUTP-containing amplicon. This degradation could be prevented by adding a bacterial UNG inhibitor (Ugi) or by setting the soak cycle to 72: C after the PCR. Finally, this contamination control procedure may not work with the LCR unless one or more of the amplimers contains uracil. However, it may be useful for the Repair Chain Reaction (RCR) or the Self-Sustained Sequence Replication (3SR) because both incorporate dTTP during the course of the amplification sequence. The present cost of the UNG procedure is \$1.50 per PCR tube.

4. 3' Ribonucleotide Amplimer: A newer alternative to the UNG procedure is the Triple CTM (Cross Contamination Control) technique shown below. In this pre-reaction protocol, the amplimers are synthesized with a RIBONUCLEOTIDE instead of the corresponding deoxyribonucleotide at the 3' position. The 3' modified amplimers replace the normal amplimers in the reaction mixture and the PCR is allowed to proceed. In a protocol similar to the UNG method, the samples for the next (second) round of PCR are then treated with either: 1) ribonuclease H or 2) strong base (OH⁻). If carryover amplicons are present, they are cleaved at the 3' end of the modified-amplimer's ribonucleotide (see Figure 4). The strand is nicked and is degraded after the first round of heat denaturation. The carryover amplicons, if present, are therefore not amplified. The manufacturer claims that this method provides a minimum of 4 orders of contamination protection. This contamination control procedure would appear to be useful for IVARs that use oligonucleotides as amplimers, which makes it suitable for PCR, LCR, RCR, and 3SR. The procedure can also function as a post-PCR control procedure. Whether this product can be effectively used for amplifying RNA-based templates, however, remains to be demonstrated. The cost for the ribonucleotide-modified amplimer technique is approximately \$0.23 to \$0.32 per PCR tube depending on the amount of amplimers used in each tube.

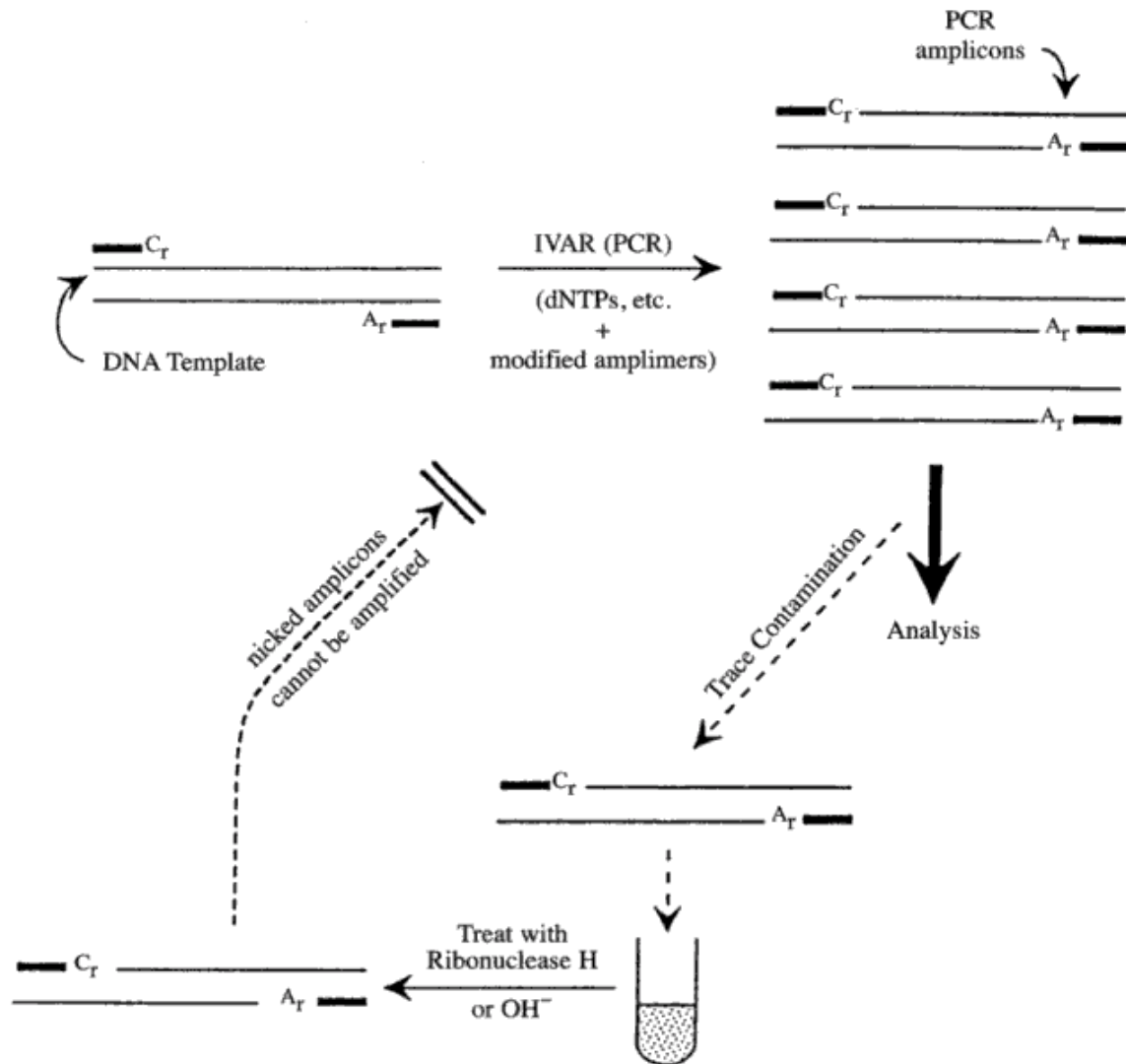


Figure 4. 3' ribonucleotide-modified oligonucleotides used for contamination control. The example amplimers contain the ribonucleotide adenine (Ar) or the ribonucleotide cytosine (Cr) at their respective 3' ends. When later attacked by ribonuclease H or OH^- , the phosphate:ribose bond is cleaved.

B. POST AMPLIFICATION CONTAMINATION CONTROL

One principle method is used for control of undesirable distribution of amplicons after the IVAR is completed. It is based upon the demonstrated sensitivity of DNA to ultraviolet light.¹² There are two different sets of photoreactions that have been developed for POST-IVAR contamination control.

1. **UV-Induced Thymine Dimers:** In this procedure, ultraviolet light (254 to 300 nm) photocrosslinks pairs of adjacent pyrimidine bases into cyclobutane-like dimers (Figure 5). The dimers are composed mostly of

thymine::thymine (TT) dimers, although a few thymine::cytosine (TC) and a rare cytosine::cytosine (CC) combinations are formed as well. Once created, these modified structures cannot be removed from the dsDNA (or ssDNA) templates because most of the thermally-stable DNA polymerases possess little or no exonuclease activity. This contamination control procedure exploits the presence of the thymine dimers (TT) since they sterically block extension of the incomplete strand when encountered by the DNA Polymerase.

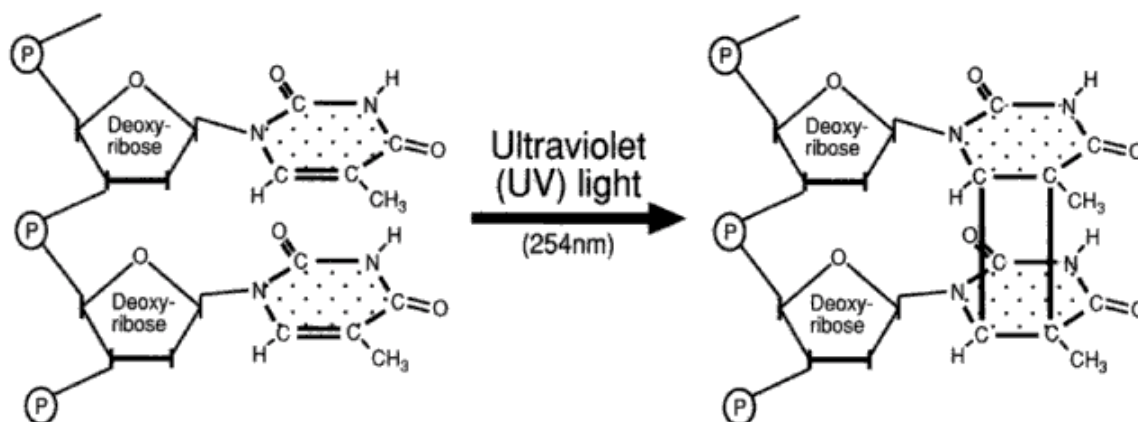


Figure 5: The formation of a thymine cyclobutane dimer in response to UV light. Similar dimers will also form between any adjacent pyrimidine pairs (e.g., CT or CC).

The extent to which the thymine dimers are formed is a statistical process dependent upon several factors, including duration of illumination and prevalence of thymines in target dsDNA. Due to some restrictions on attributes of the templates (e.g., length in bp, A:T content, frequency of adjacent thymines in DNA sequence, etc.), this technique appears to be of limited value. For example, this procedure works better for longer amplicons (e.g., ~500 bp) since the number of statistical sites for dimer formation is much greater than for short amplicons (e.g., ~100 bp).

The treatment protocol is simple. First, samples are processed by an IVAR (PCR) under normal conditions. Second, immediately after the thermal cycling reaction is completed, the unopened PCR tubes are irradiated with UV for a brief time period. Third, the samples are processed as usual [13] (Figure 6). If a UV transilluminator which emits 254 nm is used to irradiate the IVAR (PCR) tubes, the time required to effectively remove contamination has to be empirically determined, although <20 minutes may be adequate. [14,15] For DNA amplicons present in the tubes, intrastrand thymine dimers are then formed between adjacent thymines. If now accidentally introduced into a subsequent IVAR (PCR) reaction mixture, these thymine dimers inhibit extension of the single strand DNA template by sterically blocking the DNA polymerase, preventing amplification of the photoderivatized template. A potential problem of incomplete UV irradiation due to the dNTPs absorbing much of

the UV light (and hence not allowing UV crosslinking of the thymines) may be overcome by omitting the dNTPs out of the PCR master mix until after the master mix has been irradiated. [16]

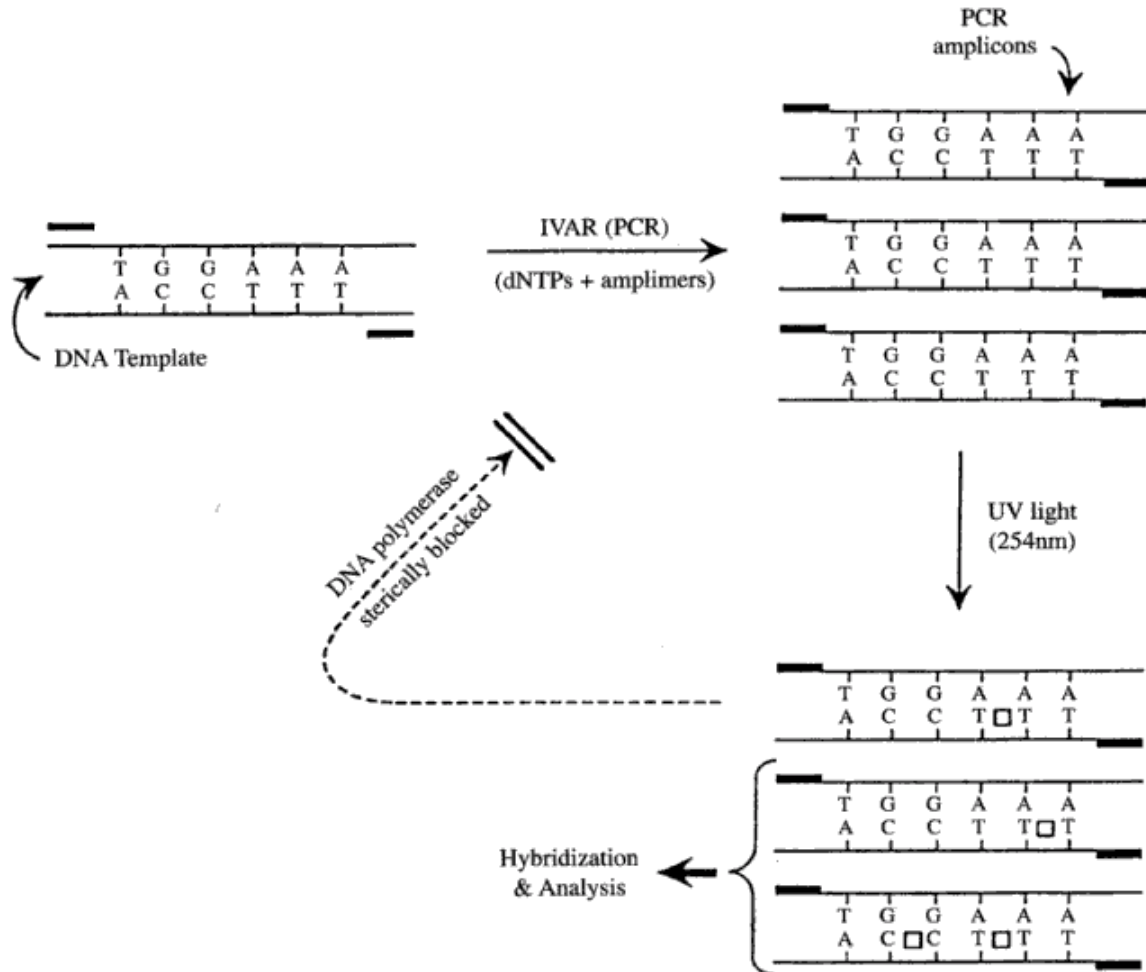


Figure 6: Contamination control procedure based upon formation of pyrimidine dimers (TT or CC) following exposure to ultraviolet light. Excision of thymine dimers is not usually performed by Taq polymerase.

Several other techniques based upon the concept of UV crosslinking have been developed. These techniques utilize UV illumination on a larger scale to expose samples and work space to eliminate contamination. One approach uses small bench top chambers (e.g., rectangular or spherical) that contain small (e.g., two 20 watt) UV lamps that shine inside a self-contained work area to provide a contamination-free work space. Another alternative is to install 254 nm UV lights into the ceiling of the laboratory where the IVAR tubes are assembled so that the entire room would be decontaminated. The latter procedure would expose the entire room (work area) overnight with 254 nm UV to provide a contamination-free

environment for use the following morning. Use of this technique would require additional safety precautions such as door interlocks on the UV circuits to protect persons working in the laboratory against UV exposure.

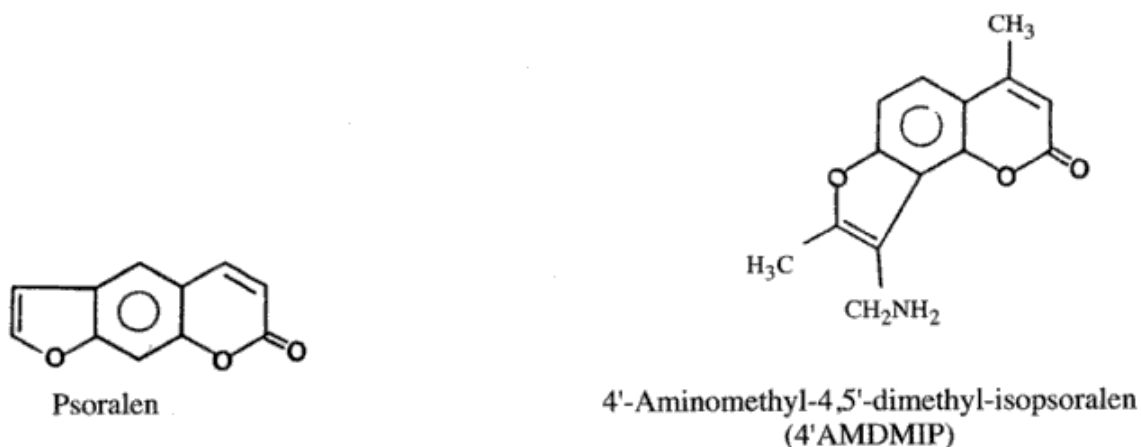


Figure 7: Structure of psoralen and related isopsoralen, 4'-AMDMIP (4-aminomethyl-4, 5-dimethyl-isopsoralen). Both are classified as furocoumarins, psoralen as a linear form and isopsoralen as an angular isomer.

2. Pyrimidine. Psoralen Photoadducts by UV: The second photoreaction is based upon the observation that a class of organic heterocyclic species ("psoralens") can undergo photometric crosslinking reactions with DNA when exposed to ultraviolet light. [17] Psoralen and isopsoralen (Figure 7) interact with dsDNA by intercalating between stacked base pairs. When illuminated with UV light, psoralen can form either monoadducts or can bifunctionally attack both strands to form interstrand derivatives. By contrast, isopsoralen (IP) can only form a monoadduct with a single adjacent pyrimidine (C or T). Although psoralen photochemistry has been exploited to chemically sterilize solutions containing DNA viruses, a method using an isopsoralen to control IVAR carryover was just recently described. [18] Several structural analogues of isopsoralen were examined for their ability to crosslink with pyrimidine bases (C and T) in DNA strands. One of the isopsoralens (4'-AMDMIP) was eventually selected and the present contamination control scheme utilizes this particular isopsoralen (Figure 8).

The isopsoralen-based contamination procedure works this way: The IVAR reagents are assembled and a mastermix is prepared. A small amount of IP is added along with the sample and the PCR is performed as described (Note: The isopsoralen is NOT incorporated into the amplicon during the main amplification reaction.) IMMEDIATELY following the IVAR (PCR), the still-sealed PCR tubes are placed inside a light-tight chamber and irradiated with UV light (300-400 nm). The isopsoralen then forms intrastrand adducts with random pyrimidine residues located along each strand. Because of its structure, isopsoralen does NOT form interstrand adducts, therefore each of the two individual derivatized DNA strands can

independently undergo hybridization reactions. [18] It should be emphasized that since the isopsoralen method is also a photoreaction, only a statistical fraction of the total number of available pyrimidines (C and T) will be modified during the illumination by UV light.

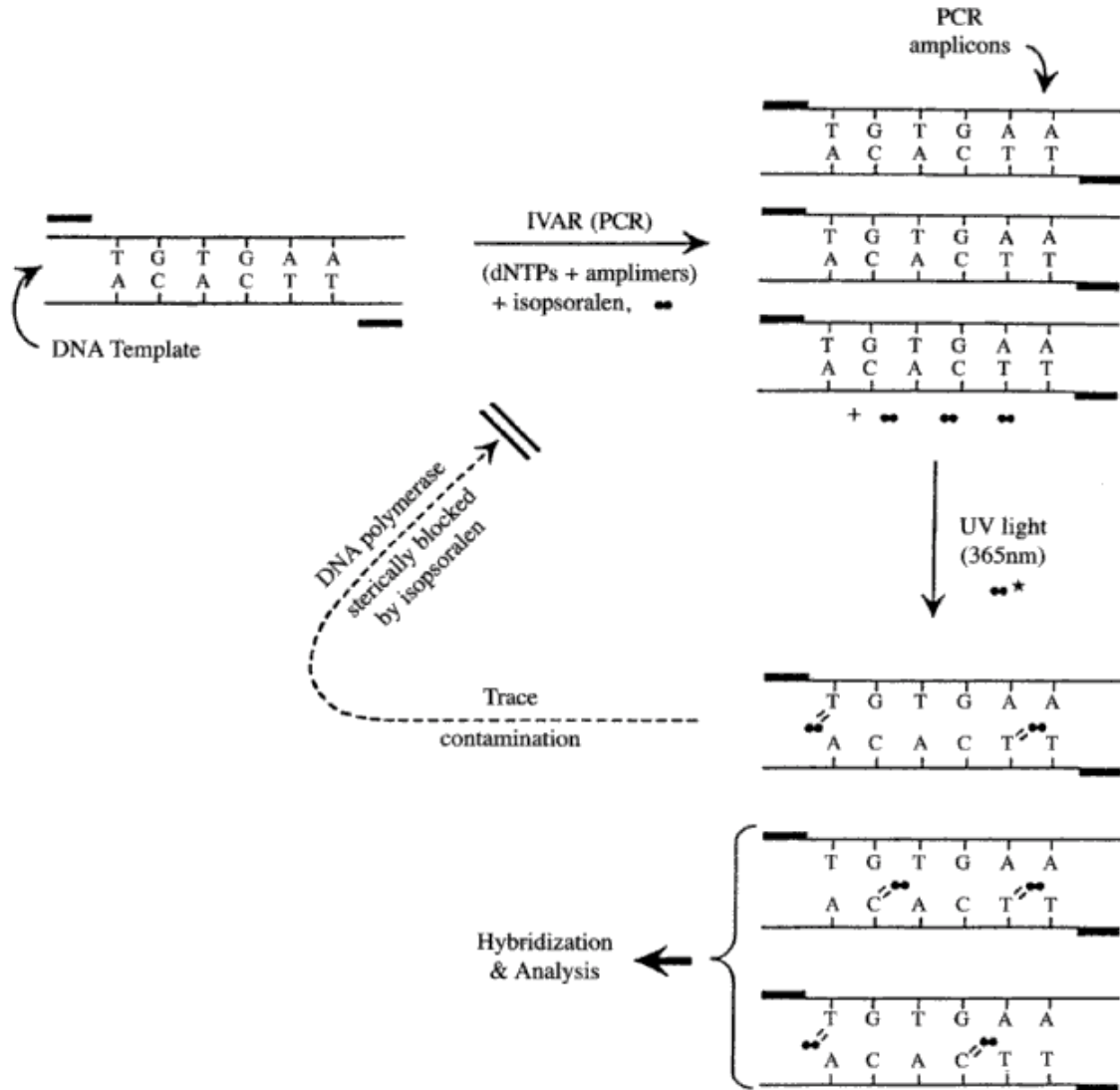


Figure 8: Contamination control technique that uses photoreaction of isopsoralen with pyrimidines (C and T). The isopsoralen species does not participate in the actual PCR reaction itself, but reacts only after it is activated by exposure to UV light. The adduct reaction is a statistical process and not all pyrimidines are modified in each separate amplicon.

A number of factors influence the extent of photoadduct formation, including intensity and wavelength of UV light, concentration of isopsoralen, amount of DNA present, and duration of UV exposure.[8] Although these variables would likely need to be optimized for each amplicon to be controlled, a consistent set of parameters could probably be chosen for most amplicons. The hybridization capability of the derivatized strands has recently been demonstrated using templates prepared from HIV. [19] The primary cost of performing this technique is the purchase of the UV Chamber (\$1495.00) and the isopsoralen reagents which are \$1.50 per PCR tube. Although this procedure appears to be useful as a contamination control technique for most of the IVAR including the PCR, LCR, RCR, and 3SR, its performance has yet to be thoroughly examined.

GENERAL CONTAMINATION CONTROL PROCEDURES

Besides using the techniques which have been described, there are some basic precautions one should observe to control contamination by IVAR amplicons. [5-7] Detailed explanations of several are listed below:

- A. **Laboratory Construction:** Once the decision has been made that an IVAR will be utilized in the laboratory, at least two separate rooms should be designated for IVAR use alone. [5-7] One room (PRE-IVAR) should be designated for preparation of IVAR reagents and samples. UNDER NO CIRCUMSTANCES should POST-IVAR tubes ever be opened or manipulated in this room. A second room for POST-IVAR analysis and manipulation should be established that is physically removed from the PRE-IVAR room.
- B. **Environmental Control:** It is preferable that these two rooms (PRE and POST) have independent environment control and not use common ductwork for air conditioning. If desired, additional modification to the laboratories can be undertaken to further control amplicon contamination. For instance, both the PRE-IVAR room and the POST-IVAR can be equipped with air-lock doors. Alternatively, PCR reagents, PCR master mixes and samples ready for cycling can be prepared in laminar flow hoods located away from other laboratory areas (i.e., "clean rooms").
- C. **Laboratory Equipment and Personnel:** Each laboratory (PRE and POST) has equipment that is unique to that laboratory (i.e., equipment that is not shared or moved between the two laboratories.) This includes small table-top centrifuges, pipettors, water baths, tube racks, etc., as well as disposable items such as pipet tips, disposable gloves, disposable pipets, etc. [5,6] Movement of persons between laboratories is also

restricted so that one individual ideally works in only one laboratory during a normal workday and does not walk periodically between both locations. Persons who do walk between these two laboratories should not wear the same labcoat or gloves in both locations. Color coded lab jackets may be useful for this purpose. It may also be necessary for individuals to wear a disposable mop cap and face mask when assembling PCR reagents. A recent report traced contamination to a laboratory person who was not protected in this manner. [4] Another report suggests that DNA amplicon contamination may also be minimized by regular use of 10% bleach on exposed surfaces such as bench counters, hoods, centrifuges, etc. [20]

- D. **Flow of Samples:** Reagents to be used for IVAR reactions and samples that may contain IVAR templates should be prepared in the PRE-IVAR laboratory ONLY. Long-term storage of these items should be restricted to this laboratory. [7] It is preferable to have the device used to facilitate the IVAR reaction ("temperature cycler") located in the POST-IVAR laboratory in case the tubes are accidentally opened following completion of the IVAR. POST-IVAR tubes should be opened ONLY in the POST-IVAR laboratory and nowhere else. All reagents or materials used for analyzing IVAR amplicon(s) should be disposed of in waste containers that are clearly labeled as originating from the POST-IVAR laboratory. These items should include agarose or acrylamide gels, blotting paper, hybridization solutions, wrapping materials, IVAR tubes, disposable pipets and wash solutions.

SUMMARY

Control of in vitro amplification reaction products is becoming an increasing concern for molecular genetics laboratories in general and for diagnostic molecular pathology laboratories in particular. What may happen within the next several years is that laboratory accreditation organizations such as the CAP (College of American Pathologists) will issue guidelines for clinical laboratories to adhere to in order to maintain certification. How robust these contamination control procedures will be and which will be more compatible with a particular IVAR is not yet known. The objective of maintaining a molecular pathology laboratory in a contamination-free state is clearly necessary. Which methods to select and how to best implement them is undoubtedly the challenge for diagnostic laboratories working in this new field.

References

1. Lo YMD, W.Z. Mehal and K.A. Fleming. "False positive results and the polymerase chain reaction." *Lancet* (1988): ii:679.
2. Kitchin PA, Z. Szotyori, C. Fromholz and N. Almond. "Avoidance of false positives." *Nature* 344 (1990): 201.
3. Hughes T, J.W.G. Janssen, G. Morgan, et al. "False positive results with PCR to detect leukemia-specific transcript." *Lancet* 335 (1990): 1037-8.
4. Pddbo S, J.A. Gifford and A.C. Wilson. "Mitochondrial sequences from a 7000 year old brain." *Nucleic Acids Res* 16 (1988): 9775-87.
5. Kwok S and R. Higuchi. "Avoiding false positives with PCR." *Nature* 239 (1989): 237-8.
6. Sambrook J, E.F. Fritsch and T. Maniatis. *Molecular Cloning: A Laboratory Manual*. 2nd ed. New York: Cold Spring Harbor Press, 14 (1989): 14.
7. Kwok S. "Procedures to minimize PCR product carryover." In: Innis MA, D.H. Gelfand, J.J. Sninsky, T.J. White eds. *PCR Protocols: A Guide to Methods and Applications*. San Diego: Academic Press, (1990): 142-5.
8. Persing DH. "Polymerase chain reaction: trenches to benches." *J Clin Micro* 29 (1991): 1281-5.
9. Williams, JF. "Optimization strategies for the polymerase chain reaction." *BioTechniques* 7 (1989): 762-7.
10. Longo MC, M.S. Berninger and J.L. Hartley. "Use of uracil DNA glycosylase to control carryover contamination in the polymerase chain reaction." *Gene* 93 (1990): 125-8.
11. Thornton CG, J.L. Hartley and A. Rashtakian. "Utilizing uracil DNA glycosylase to control carryover contamination in PCR: Characterization of residual activity following thermal cycling." *BioTechniques* 13 (1992): 80-82.
12. Gordon LK and W. A. Haseltine. "Quantitation of cyclobutane pyrimidine dimer formation in double- and single-stranded DNA fragments of defined sequence." *Rad Res* 82 (1982): 99-112.
13. Cimino GD, K. Metchette, S.T. Isaacs and Y.S. Zhu. "More false positive problems." *Nature* 345 (1990): 773-4.

14. "Controlling PCR Contamination." Fotodynamics Newsletter New Berlin: FOTODYNE 6, October (1990): 1-2.
15. Sarkar G and S.S. Sommer. "Shedding light on PCR Contamination." Nature (1990): 343:27.
16. Fronthingham R, R.B. Blitchinton, D.H. Lee, R.C. Greene and K.H. Wilson. "UV absorption complicates PCR decontamination." BioTechniques 13 (1992): 208-210.
17. Cimino GD, H.B. Gamper, S.T. Isaacs and J.E. Hearst. Ann Rev Biochem 54 (1985): 1151-93.
18. Cimino GD, K.C. Metchette, J.W. Tessman, J.E. Hearst and S.T. Isaacs. "Post-PCR sterilization: A method to control carryover contamination for the polymerase chain reaction." Nucleic Acids Res 19 (1991): 99-107.
19. Isaacs ST, J.W. Tessman, K.C. Metchette, J.E. Hearst and G.D. Cimino. "Post-PCR sterilization: development and application to an HIV-1 diagnostic assay." Nucleic Acids Res 19 (1991): 109-16.
20. Prince AM and L. Andrus. "PCR: How to kill unwanted DNA." BioTechniques 12 (1992): 358-59.

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Biography of Theodore E. Mifflin

Theodore E. Mifflin, Ph.D., DABCC, is an Associate Professor of Pathology at the University of Pittsburgh Medical Center, Pittsburgh, PA. He received a B.S. in Chemistry from Weber State University in Ogden, Utah and later a Ph.D. in physical biochemistry from Utah State University in Logan. He was a postdoctoral fellow at the University of Virginia Medical Center from 1983 to 1986 and a Research Assistant Professor at the University of Virginia from 1986 to 1990. In 1991 he accepted his current position at the University of Pittsburgh. Dr. Mifflin directs a molecular genetics laboratory that is part of the Molecular Diagnostics Division within the Department of Pathology. He is also a consultant to Molecular BioProducts, Inc.

About Molecular BioProducts, Inc.

Founded in 1978, Molecular BioProducts, Inc., has been a manufacturer of innovative products designed to eliminate contamination concerns for researchers performing PCR. Now, with a line of patented and proprietary products that are recognized and respected throughout the industry, MBP has become the leader in contamination control products for molecular biology. To further assist you and your lab, MBP is pleased to provide this overview to contamination control in the PCR laboratory. It was the comments and questions of researchers around the world that prompted us to contact Dr. Theodore Mifflin and develop this manual. In it, Dr. Mifflin shares his many years of experience and expertise in a concise and informative way and for this, we owe many thanks.

Our commitment to helping researchers prevent contamination is represented in our line of quality products and in the publication of this booklet, each of which can be used to increase the accuracy and reliability of your research. The valuable information in this report can help solve many of the common problems associated with cross-contamination, but we invite you to contact us with any additional questions or problems that you are facing in the laboratory. We thank you for your continued support and look forward to serving you in the future.