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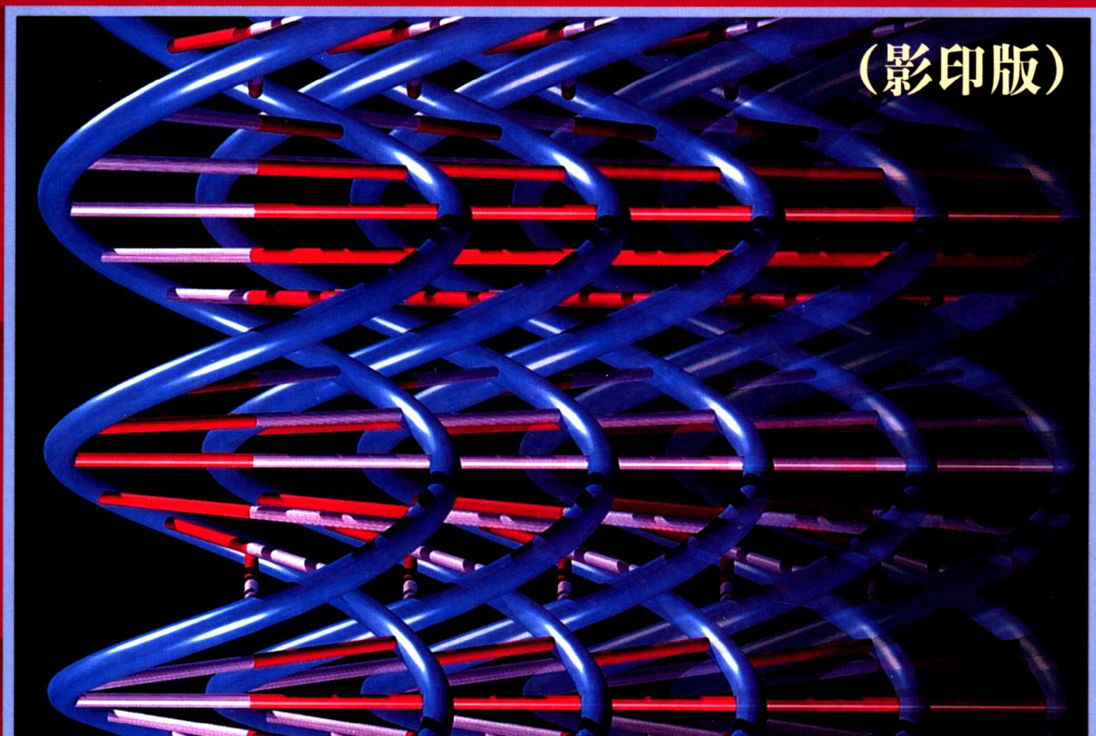
PCR技术实验指南

PCR Primer: A Laboratory Manual

第二版

〔美〕C.W.迪芬巴赫　G.S.德维克斯勒 主编



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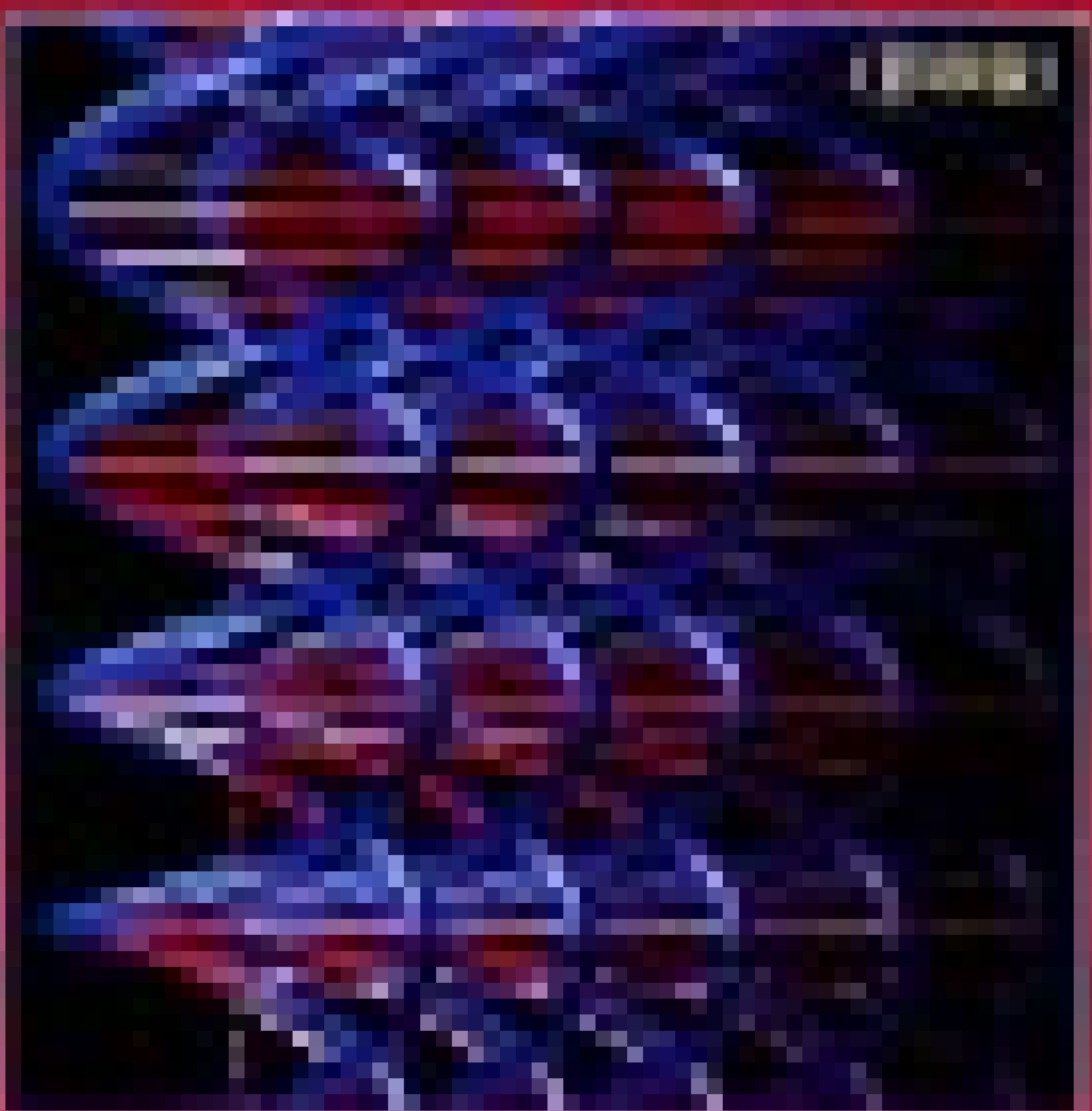

PCR 技术实验指南

(第二版)

〔美〕C.W. 迪芬巴赫　G.S. 德维克斯勒　主编

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内 容 简 介

聚合酶链反应(PCR)自发明至今的20年来,经过不断的开发和改进,现在已经广泛应用在诸多领域,是分子生物学、临床诊断、法医检验等实验室的常规技术。

本书是美国冷泉港实验室出版社 *PCR Primer: A Laboratory Manual* 第二版的影印本,是第一版的全面更新和升级。本书由从事PCR研究的专家们共同研讨和撰稿,是一部最新、最权威的PCR技术实验手册。其内容从最简单的PCR操作到最新的技术改进,从最基本的PCR技术到各种奇思妙想的PCR应用技巧,无所不至,令读者目不暇接、备受启迪。具体内容包括PCR导论、样品的制备、引物设计、PCR产物检测(定量和分析)、RNA样品的PCR、PCR介导的克隆、PCR法制备突变体、其他扩增技术等。每个操作都配有疑难解析和完整的参考文献以供答疑、检索。

本书是《分子克隆实验指南》的姊妹篇,可供从事分子生物学、遗传学、细胞生物学、生物化学以及医学各个领域研究的科研与教学人员参考,既是实验室必备的工具书,也是研究人员开阔眼界、拓展思路的不可不读之经典。

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Preface

THE POLYMERASE CHAIN REACTION HAS BECOME one of the most widely used techniques in molecular biology, and it is very rare nowadays to find a molecular laboratory without a PCR machine. This technique has facilitated many traditional cloning experiments and has made possible procedures that were previously impossible, usually because of a lack of adequate amounts of nucleic acid. Coupling the information available from genome sequencing with the ability to synthesize DNA at will, PCR provides investigators with tools to address nearly every question in molecular biology. Since the first edition of *PCR Primer: A Laboratory Manual,* there have been some significant advances, as well as incremental improvements in technology and methods. This second edition has captured these advances and integrated them with the tried-and-true methods, which have been updated.

Our thanks and appreciation go to the authors of the chapters, who provided us with manuscripts and were patient with our edits to ensure continuity and consistency throughout the book. We thank the wonderful staff at the Cold Spring Harbor Laboratory Press that has been an essential part of this process: Inez Sialiano has cheerfully kept us on track and on schedule, and Patricia Barker has smoothed out the production process. As with the first edition, this book would not have been possible without Judy Cuddihy, whose advice, guidance, and assistance were essential for the completion of this project. Now it is up to you, the reader of this book, to apply what you know to advance knowledge and make the world a better place.

C.W.D.
G.S.D.

SECTION 1

INTRODUCTION TO PCR

The polymerase chain reaction (PCR) is a deceptively simple technique. The process of repetitive bidirectional DNA synthesis via primer extension of a specific region of nucleic acid is so fundamental in design that it can be, and has been, applied in seemingly endless ways to solve problems in nearly every field of science. This basic description implies several critical processes: (1)that the target DNA is single stranded, and that conditions occur for target sequence-specific annealing of the primers; (2) that the primers are extended, converting the target DNA from single stranded to double stranded; and (3) that the double-stranded DNA denatures, beginning the process anew.

These processes are functionally programmed into the PCR machine so that each reaction proceeds through each step in an orderly manner. DNA denaturation occurs when the reaction is heated to 92–96°C. The time required to denature the DNA depends on its complexity, the geometry of the tube, the thermal cycler, and the volume of the reaction. For DNA sequences that have a high G+C content, the addition of betaine as described in "PCR Amplification of Highly GC-rich Regions" (p. 43) has been reported to improve the yield of the PCR.

After denaturation, the oligonucleotide primers hybridize to their complementary single-stranded target sequences. The temperature of this step depends on the length and base composition of the primers. In "PCR Primer Design" (p. 61), methods are described to select primers that have the maximum likelihood of success. Because primers hybridize nearly instantaneously to their target sequence at the proper annealing temperature, this step in the cycle can be manipulated to improve the yield and specificity of the PCR, as described in "Optimization and Troubleshooting in PCR" (p. 35).

The last step is the extension of the oligonucleotide primer by a thermostable polymerase. Traditionally, this portion of the cycle is carried out at 72°C. The time required to copy the template fully depends on the length of the PCR product. The time considerations as related to the length of the PCR product are described in "Tips for Long and Accurate PCR" (p. 53).

The major strength of PCR is its ability to specifically detect a vanishingly small amount of target nucleic acid sequence, even in the presence of a vast excess of other nucleic acid. Whether one is a novice or an expert, the amplification capacity of PCR can also cause tremendous problems.

CONTAMINATION ISSUES

The most serious issue with the widespread use of PCR is the contamination of new reactions with amplified product from old reactions or target nucleic acids. This can occur during several steps prior to the actual amplification reaction. The chapters "Dealing with Carryover Contamination in PCR: An Enzymatic Strategy" (p. 15) and "Setting up a PCR Laboratory" (p. 5) provide specific protocols to reduce contamination, and the latter chapter discusses how to integrate these methods into good laboratory practices and improved laboratory design.

To start at the beginning with laboratory design is a luxury that most of us cannot afford. In this regard, it is never too late to implement some basic procedures to minimize contamination. These are (1) physical separation of pre- and post-PCR activities; (2) separation of standard molecular biology from pre-PCR; (3) dedication of equipment and supplies solely to the pre-PCR area; and (4) judicious use of positive and negative controls.

Another critical step in contamination control is the sensible handling of reagents in the pre-PCR area. Rules for the safe handling of reagents and supplies in the PCR laboratory include:

1. Prepare solutions free from contaminating nucleic acids and/or nucleases (both DNases and RNases).
2. Use the highest-quality water in all PCR reagents. Water should be freshly distilled/deionized, filtered using a 0.22-micron filter, and autoclaved. USP-certified water that has been filtered and autoclaved is also sufficient for use in PCR. Aliquot the water so that a new bottle is used for each set of experiments.
3. Sodium azide, 0.025%, should be added to any reagent stored at room temperature. This does not inhibit the amplification reaction.
4. For consistency and reproducibility, all reagents should be made up in large volumes, then tested and, if acceptable, dispensed into single-usage volumes for storage.
5. Use only disposable, sterile bottles and tubes for all sample and reagent preparation.
6. Test all new sample preparation reagents before using on new, valuable specimens.
7. Carefully store all pipettes used in sample preparation and pre-PCR when not in use. Ziploc bags are good for this purpose.
8. Wear gloves at all times when preparing reagents, handling samples, and setting up reactions. The handling of the reaction after amplification must be in a different area of the laboratory.
9. Dedicated lab coats are required for both the pre-PCR and post-PCR areas. Keep the experimental flow from sample preparation, to pre-PCR, into post-PCR analysis.

STANDARD PCR

With all the improvements in PCR, what is currently considered standard PCR? PCR amplification of a template requires two oligonucleotide primers, the four deoxynucleotide triphosphates (dNTPs), magnesium ions in molar excess of the dNTPs, and a thermostable DNA polymerase to perform DNA synthesis. The target region to be amplified should be between 150 and 400 nucleotides. The quantities of oligonucleotide primers, dNTPs, and magnesium, and the final volume, may vary for each specific application.

The components of a standard PCR protocol using *Taq* DNA polymerase are as follows:

10x Enzyme-specific reaction buffer:
- 10–50 mM Tris-HCl, pH 7.5–9.0
- 6–50 mM KCl or $(NH_4)_2SO_4$
- 1.5–5.0 mM $MgCl_2$ or $MgSO_4$

0.2 mM of each dATP, dGTP, dCTP, and dTTP
0.1–1.0 μM of each oligonucleotide primer
2.0–2.5 units of a thermostable DNA polymerase
Nucleic acid template, 10^2–10^5 copies
Distilled water to 50 μl

CHOICE OF REAGENTS AND PROCEDURES

Depending on the application, *Taq* polymerase may not be the best choice. In the chapters "High-Fidelity PCR Enzymes" (p. 21) and "Tips for Long and Accurate PCR" (p. 53), the case is made for using enzymes from other thermophiles or mixtures of enzymes to deliver consistent results without risk of the appearance of mutation in the product. The methods proposed by Barnes in "Tips for Long and Accurate PCR" are sufficiently robust to easily amplify target sequences from 300 nucleotides up to very long products in the kilobase range.

For any of these reactions to work, there is a need to establish conditions for selective amplification of the target sequence. The determination of the temperatures to be used at each step in the PCR cycle and the length of the primer extension step are dependent on the base composition of the target sequence, the length of amplicon, and the annealing temperatures of the primers. Methods to define conditions for amplification are covered in "Optimization and Troubleshooting in PCR" (p. 35).

Another method that has proven very useful in the optimization of PCR is the use of the hot-start procedure. Hot-start PCR is defined as any method that prevents the primer extension of inappropriately bound primers. Although mispriming can be avoided in some situations by paying close attention to the primer design and by selecting the correct annealing temperature for the amplification reaction, it is also necessary to avoid having the components of the reaction at a temperature lower than the annealing temperature chosen for the specific amplification. This can be easily accomplished with hot-start methods. There are a number of recommended methods for this, including uracil-*N*-glycosylase and dUTP, a wax barrier, a wax bead impregnated with magnesium, an anti-*Taq* DNA polymerase monoclonal antibody, or the purchase of enzymes precomplexed with a heat-labile inhibitor. With all of these methods, after heating the reaction to 92°C for the first time, all the reaction components mix, and DNA synthesis occurs only from accurately hybridized primers. Hot-start methods are described in three chapters: "Dealing with Carryover Contamination in PCR: An Enzymatic Strategy" (p. 15); "High-Fidelity PCR Enzymes" (p. 21); and "Optimization and Troubleshooting in PCR" (p. 35); and "Amplification of RNA: High-temperature Reverse Transcription and DNA Amplification with a Magnesium-activated Thermostable DNA Polymerase (p. 211). Another important consideration in the optimization of PCR is the overall GC content of the target DNA. PCR depends on the complete denaturation of the target DNA each cycle. As discussed in "PCR Amplification of Highly GC-rich Regions" (p. 43), special methods are often needed to get the DNA to amplify.

With the best enzymes and buffers, PCR cannot proceed without quality template nucleic acid or PCR primers. Nucleic acid purification is such an important topic that it has a separate section. The "PCR Primer Design" chapter (p. 61) covers all aspects of design including the construction of Taqman and Scorpion probes for real-time PCR.

CONTROLS

Even with the best reagents, primers, and supplies, proper controls must be performed to test for PCR performance and presence of DNA contamination. As a rule, there should be a panel of negative, weak, and strong positive control samples to assay the efficiency and cleanliness of the sample preparation and pre-PCR process. The negative controls that are run alongside each set of samples should be constructed to assay for sample-to-sample contamination as well as for contamination with PCR products. The negative controls should include all reagents except the template DNA.

The amount of target DNA in positive controls should be minimized for two reasons. To test for robust amplification, only 10^1–10^4 copies of the target sequence should be included in the reaction to serve as a valid control. Second, by limiting the amount of DNA, there is less chance of this material serving as a source of contamination. When setting up the reactions, always pipette the template in the tubes designated as positive controls last.

PCR-BASED SEQUENCING

Finally, PCR has helped to revolutionize DNA sequencing. The ability of PCR-based methods to selectively amplify and sequence in the absence of radioactivity, with limited amounts of template, which has or has not been cloned into a vector, helped provide the systems to accomplish the Human Genome Project. A robust method is provided in "Nonradioactive Cycle Sequencing of PCR-amplified DNA" (p. 75).

The chapters in this section lay the foundation for harnessing the full capacity of PCR. Careful attention to laboratory practices and incorporation of contamination control methods will make PCR a seamless part of your laboratory.

1 Setting Up a PCR Laboratory

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Development of the polymerase chain reaction (PCR) as a basic component of the molecular biology laboratory has occurred very rapidly from its inception in 1985. Since then, more than 15,500 articles have been published in which this technique was used. (See Table 1 for additional information sources for PCR.) As PCR became more widely used, scientists rapidly learned more about it and, as a result, learned that the PCR had its strong points and its deficiencies. Very quickly, PCR demonstrated its power to amplify very small amounts (e.g., a single copy) of template nucleic acid and to amplify different nucleic acids (e.g., DNA and RNA). At the same time, laboratory personnel learned that this biochemical reaction had a unique deficiency; namely, a strong susceptibility to contamination from its own product. Early experience with the PCR soon showed that additional precautions were needed (Lo et al. 1988; Kwok and Higuchi 1989). This chapter is devoted to establishing a PCR laboratory whose operations will give reliable and contamination-free results.

CONTAMINATION ISSUES

PCR contamination remains an issue for laboratories performing forensic procedures and detection of infectious agents (Pellett et al. 1999; Scherczinger et al. 1999). There are a number of approaches to control of PCR contamination, and the degree of stringency that is required in a laboratory is often determined by the assay being performed.

TABLE 1. Listing of Internet Web sites and URLs for PCR information

#	Web site (URL)	Comment
1.	http://highveld.com/pcr.html	PCR Jump station (Web portal)
2.	http://www.dnalc.org/resources/BiologyAnimationLibrary.htm	Downloadable animated video of PCR (PC or MAC)
3.	http://ncbi.nlm.nih.gov	GenBank site for checking amplimer (primer) sequences
4.	http://www.mbpinc.com (then select "Tech Reports" for monograph)	PCR contamination monograph
5.	http://info.med.yale.edu/genetics/ward/tavi/PCR.html	Guide to multiplex PCR

Amplicon Aerosol

The single most important source of PCR product contamination is the generation of aerosols of PCR amplicons that is associated with the post-PCR analysis. Methods for eliminating this aerosol range from physical design of laboratories and use of specific pipettes to chemical and enzymatic approaches. The choice of method is often dependent on the frequency of amplification of a target amplicon and the relative amounts and concentrations of the amplicons created by the PCR.

Target Template Contaminants

In addition to post-PCR contamination, the target template itself can be a source of contamination. For example, DNA templates are typically more troublesome as contaminants because they are more stable than RNA targets. Detection of infectious agents typically demands the most stringent contamination efforts, whereas detection of other targets, such as those from inherited disease, may require less contamination control. Regardless of the template to be detected, good laboratory practices should be followed (Kwok and Higuchi 1989) (see details below).

Real-time PCR Systems

PCR systems exist that provide direct measurement of amplicon accumulation during the reaction. These real-time PCR systems offer an alternative approach to the traditional post-PCR analysis methods. From a contamination control perspective, the collection of data during the amplification reaction by using a fluorescence-based detection system eliminates the need to handle the sample. Thus, when these PCRs are completed, the detection and analysis are complete, the reaction tubes remain sealed, and there is no amplicon escape. Arranging the PCR laboratory to perform these homogeneous (or real-time) PCRs requires a different approach, which is addressed later in this chapter. Because the major use of PCR as a laboratory tool still depends on a separate post-amplification manipulation, this approach is the one primarily addressed.

CONTAMINATION PREVENTION APPROACHES IN THE PCR LABORATORY

The PCR laboratory typically is involved with activities that include sample preparation, PCR reaction assembly, PCR execution, and post-PCR analysis. These activities are summarized in Figure 1. When arranged in this linear fashion, these activities can be collected into two major groups, the pre-PCR activities (sample preparation and PCR preparation) and the post-PCR activities (PCR execution and analysis).

Use of the PCR for research and diagnostic purposes requires that some additional procedural limitations be observed so that the reaction yields valid results. As awareness of the PCR's susceptibility to contamination became known, Kwok and Higuchi (1989) presented some additional guidelines for researchers using the PCR. Consistently observing these guidelines is essential for successfully operating a PCR laboratory on a long-term basis. They form part of a network of protocols focused on maintaining a PCR laboratory in a contamination-free condition.

Contamination can arise from several different sources, such as previous amplification and purification of plasmid clones, repeated isolation of template (genomic) nucleic acids, and previously amplified molecules ("amplicons"). Although most attention in a PCR lab-

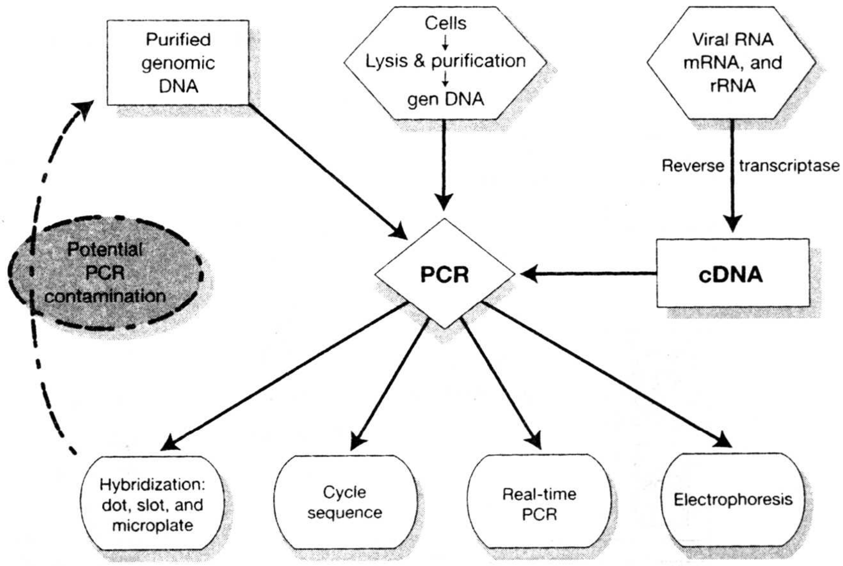

FIGURE 1. Outline of sample processing and analysis in a PCR laboratory.

oratory is focused on the last category of contamination, the other two sources should not be ignored. A prudent approach to controlling their contamination is to segregate the more standard recombinant DNA activities into separate areas of the laboratory and, in particular, to limit the performance of PCR activities to its own area.

For PCR amplicon contamination, it is the control and removal of the PCR amplicons that form the basis for the contamination control program. When PCR is used in research laboratories, either a greater variety of templates or amplifications of a specific template will be studied or manipulated and, thus, controlling amplicon contamination may be less challenging. In a diagnostic laboratory, there can be more opportunities for PCR contamination due to the repeated analysis of selected templates and the fact that PCR assays may be performed at or near the detection limit of PCR. The last possibility is especially demanding and thus requires a much more rigorous approach to controlling PCR contamination.

The essential parts of this contamination control program include space and time separation of pre- and post-PCR activities, use of physical aids, use of ultraviolet (UV) light, use of aliquoted PCR reagents, incorporation of numerous positive and negative or blank PCRs (H_2O substituted for template), and use of one or more various contamination control methods that use chemical and biochemical reactions. The underlying theme in these actions is the recognition that amplicon contamination cannot be seen, felt, or a priori detected before it happens. Use of consistent, careful technique coupled with liberal incorporation and monitoring of PCR blanks will ensure a vigilant, proactive approach to PCR contamination.

Space and Time Separation

As illustrated in Figure 1, the main source of the feedback contamination is the amplicons generated by the previous PCR. By separating the source of the amplicons' (e.g., post-PCR) activities from the pre-PCR activities, the potential for contamination is significantly reduced. This separation is best illustrated by separating the facilities in space, so that there are two rooms where these activities occur (Fig. 2). If this is not achievable, different areas designated for sample preparation and PCR setup can be located away from the area for

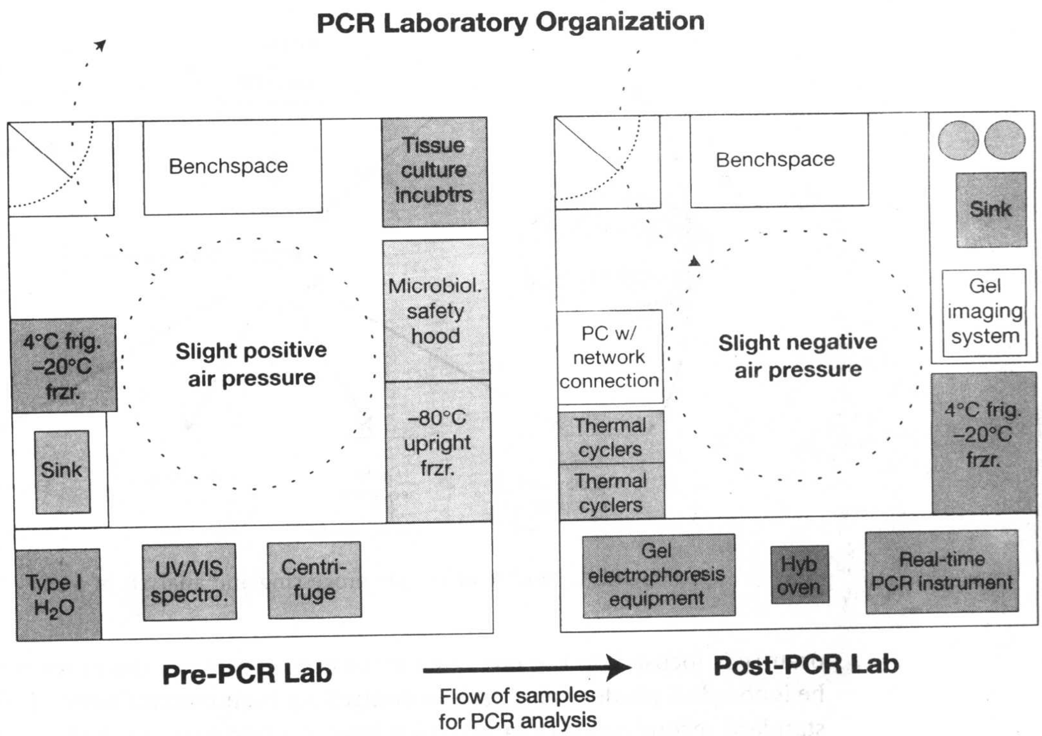

FIGURE 2. Organization of a PCR laboratory with separate pre- and post-PCR rooms.

post-PCR analysis. If all activities are to be performed in a single room, sample preparation should occur inside a laminar flow hood, preferably equipped with a UV light. The walls of the hood should be wiped with a fresh (or freshly made) 10% bleach solution (1 part regular bleach: 9 parts water) before processing samples or preparing PCR samples. Waste materials that contain PCR amplicons should not be allowed to accumulate in an area that is also frequented by other personnel who may be eventually involved with template isolation and purification. Additionally, the laboratory should consider establishing a daily schedule for performing PCR. Sample preparation and pre-PCR should be morning activities. Once completed, the pre-PCR supplies and equipment can be stored, and the afternoon can be devoted to the post-PCR analysis.

Laboratory Space Arrangement

As mentioned above, the ideal arrangement of the PCR facility is to have the pre- and post-PCR areas located in separate rooms (see Fig. 2), each with dedicated resources. A source of deionized water needs to be present in both rooms, as well as dedicated centrifuges, storage freezers/refrigerators, and storage of supplies. Even telephones, computers, and other electronic communications should also be dedicated.

Rarely is laboratory space allocated strictly for PCR; it often must be shared with other procedures. In a research laboratory, cell culture and other biochemical protocols such as analysis for enzymes and other biochemical species, protein and nucleic acid isolation, cloning, transfection, and purification may also occur. Thus, PCR protocols may likely be integrated into the laboratory's operation with consequent need to share facilities and bench space.

In the case of the diagnostic laboratory, a much more limited menu of activities occurs that is focused primarily on sample isolation and processing, although other possible activities such as cloning or sequencing can happen as well. There is usually a greater demand to detect and quantify low amounts of template in biological samples in the diagnostic laboratory, and this places a greater need on preventing amplicon contamination proactively.

A research institution may be unable to have separate facilities unless an arrangement can be developed for a "core" PCR setup facility, with each laboratory performing its own PCR and subsequent amplification. Where there is a requirement for detection of very low amounts of template nucleic acid (DNA or RNA), separate facilities offer the greatest likelihood that contamination-free results can be obtained over a long time frame.

An alternative to two-facility arrangements is found in real-time PCR methods. Because the results of each PCR are provided throughout the reaction, there is no need to open the PCR containers, and these can be discarded while still sealed. At least one instrument manufacturer is developing a completely self-contained automated system (the AmpliPrep: Taqman from Roche Molecular Systems, Alameda, CA) that would eliminate the need for two separate rooms. However, there may be situations where the contents of individual tubes and capillaries created during real-time PCR analysis need to be analyzed independently, and so these containers would need to be opened. This should happen in a different room from where the real-time PCRs were set up and performed.

Equipment in PCR Laboratories

To ensure that pre-PCR and post-PCR events remain separated, each room must have its own separate set of equipment, including pipettors, reagents, pipettor tips, racks, and so forth. Moreover, these items should not leave the area to which they are assigned. Each should be labeled as to location and used in that location only. Lab coats should be dedicated for both areas as well. Because pipetting forms the basis for most PCR analysis, each area needs its own dedicated pipettors that are never exchanged between work areas. To assist with this, color-coded pipettors (e.g., green for pre-PCR work, red for post-PCR work) can be used. When pre-PCR pipettors and tips are not in use, they should be stored in airtight bags to keep them clean. Reactions should be constructed using master mixes, and the template should always be added last using positive displacement tips to prevent pipettors from becoming cross-contaminated while pipetting samples that contain template. These types of pipettors and tips are available from several sources and can be purchased in sterilized packs. It is important to remember that barrier tips cannot be autoclaved.

Pre-PCR Activities

The definition of pre-PCR is the protocols and equipment required for the isolation of nucleic acid and the assembly of the reaction to amplify the samples. During the last 10 years, there has been much progress in developing devices that perform these activities in an automated fashion. Most PCR laboratories still perform these tasks using manual procedures.

What is the minimum needed to equip a PCR laboratory for sample preparation, PCR reagent preparation, and PCR assay setup? Because most of the activities revolve around pipetting of liquids, these activities should be examined most closely; in particular, the manual pipettors and pipette tips. As discussed previously, positive displacement tips or barrier methods should be used to pipette the template into the reactions as the last step.

There is a risk of creating aerosols in the preparation for RNA and DNA templates. If a large number of specimens of one type are processed on a routine basis, the laboratory may wish to treat this method with care and perform it in a hood or biosafety cabinet (Fig. 2). Because of the effectiveness of ultraviolet light (UV) for amplicon control, use of UV inside the cabinet prior to sample preparation or PCR reagent preparation is advisable. Alternatively, any one of a number of small, benchtop-size cabinets that use UV irradiation can also be utilized. These are dedicated to PCR use and are large enough to contain several pipettors, racks, and some reagents.

Environmental Considerations

- *Air handling.* For extremely high-performance PCR laboratories that will be involved with detecting very-low-prevalence DNA or RNA molecules (e.g., infectious disease agents in clinical samples), additional measures may be necessary to prevent contamination from the air being recirculated between the pre- and post-PCR laboratories. In this case, the air handlers need to be separate and the air pressure individually adjusted in each laboratory. In the pre-PCR laboratory, there should be a slight positive pressure compared to the air in the connecting hallway. The post-PCR laboratory, in contrast, should be at slightly reduced pressure to pull air in from the outside and thereby prevent escape of amplicons from the completed PCR samples being analyzed inside the lab (Fig. 2). Finally, the air handlers for the pre- and post-PCR laboratories need to be connected to separate air ducts, and each must lead to a separate location for exhaust.
- *UV irradiation.* It is possible to exploit further the sensitivity of nucleic acid to UV by using UV to sterilize the entire pre-PCR laboratory. This can be done by having UV lights placed in the ceiling fixtures and connecting their activation to a lock-out mechanism on the exit door so they only illuminate when the last person in the lab closes and locks the external lab door. If this type of hardware is installed, it must be accompanied by a ventilation system to eliminate the UV-generated ozone and a rigidly enforced schedule of monitoring the performance of the UV bulbs. These light fixtures accumulate a residue arising from the precipitation of oxidation products on the glass of the bulb. If this is not removed monthly, the UV system is not effective.
- *Protective clothing.* To further prevent PCR amplicons from leaving the post-PCR lab, each investigator should have a dedicated post-PCR lab coat. Additionally, each investigator should have a general molecular biology lab coat and a separate coat for pre-PCR. In extreme cases, a disposable gown and booties should be worn.
- *Adhesive paper at lab entrances.* This approach effectively prevents trace amounts of dust and debris from entering the laboratory. It is a rather expensive approach to controlling contamination, but may be worth the expense for selected applications.

Sterilization of Reagents

Because PCR laboratories perform some molecular biology methods that require sterile reagents, some may need to be autoclaved. The single most critical reagent is water. Sterile USP water can be quickly converted to PCR water by filtering it through two 0.45-micron nitrocellulose filters. These filters have a very high binding capacity for nucleic acid and proteins. If the laboratory is involved in amplification of very small quantities of bacterial DNA, the USP water should be autoclaved separately from all other reagents before filtration. In general, reagents and solid items destined for the pre-PCR lab should be autoclaved separately from other supplies. It is important to note that spent tissue culture fluids, bac-