Molecular Biology Problem Solver: A Laboratory Guide. Edited by Alan S. Gerstein

ISBNs: 0-471-37972-7 (Paper); 0-471-22390-5 (Electronic)

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Preparing for Success in the Laboratory

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THE PROJECT

If You Don't Define the Project, the Project Will Define You

One of the first and toughest questions researchers must answer to foster success in the lab is: What do I have to accomplish? This requires you to understand your purpose to the larger task at hand. If your research is self-directed, the answer will most likely differ from that for someone working as part of a team effort or answering to an immediate supervisor or experimental designer. Ask them (or yourself) what the ultimate goals are and what constitutes a successful outcome. Establish what constitutes compelling evidence. By projecting ahead it becomes much easier to characterize the nature of the desired outcome.

This approach allows for problem reduction and reasonable task planning. The greatest mistake one can make is to react hastily to the pressures of the research by jumping in unprepared. By starting with the big picture, the stage is set for working backward and reducing what might otherwise appear to be a daunting undertaking into a series of reasonably achievable tasks. This exercise also establishes the criteria for making the many decisions that you will face during the course of your work.

Which Research Style Best Fits Your Situation?

Certain decisions will have a profound impact on the nature and quality of your efforts. Some scientists favor deliberate attention to detail, careful planning and execution of each experiment. Others emphasize taking risks, skipping ahead and plunging in for quick results. You might want to consider which approach would best satisfy your superior(s) and colleagues. Each of these "styles" has its benefits and risks, but a well-balanced approach takes advantage of each. Sometimes it is essential to obtain a quick answer to a question before committing a substantial amount of time to a more diligent data-collecting phase. Be sure everyone involved is in agreement and then plan your activities accordingly.

Do You Have the Essential Resources?

Evaluate your circumstances with a critical eye. Look at your schedule and that of your collaborators. Is everyone able to devote the time and energies this project will demand with a minimum of distractions? Check your facilities; do you have access to the materials and methods to do the job? Do you have the support

of the decision-makers and budget managers for the duration of the work?

Whether or not problems were uncovered, share your findings with your director and collaborators; the objective of this phase is to build a consensus to proceed with no further changes.

Expect the Unexpected

How flexible is your research plan? Have you allowed yourself the freedom to adapt your strategy in light of unanticipated outcomes? This happens frequently and is not always bad news. Unexpected results might require slowing down the process or stopping altogether until a new path can be selected. Perhaps whole elements of the work might be skipped. In any case you should plan on midcourse corrections in your schedule. You can't always eliminate these redirections, but if you plan for them, you can avoid many unnecessary surprises. There are likely to be multiple paths to the desired outcome. If the unexpected occurs, consider categorizing problems as either technical or global. Technical problems are usually procedural in nature. The data obtained are either unreliable or untenable. In the former case the gathering of data may need to be repeated or the procedure optimized to the new conditions in order to increase data reliability. In the later case the procedure may prove to be inadequate and an alternative needs to be found. A global problem is one in which reliable data point you in a direction far removed from the original plan.

Technical problems are ultimately the responsibility of the principal investigators, so keep them informed. They might provide the solution, or refer you to another resource. Sometimes these problems can take forever to fix, so an upper limit should be agreed upon so that long delays will not be an unpleasant surprise to the other participants. Delays can be the source of much resentment among team members but should be considered an unavoidable consequence of research.

Global problems might require more drastic rethinking. The challenge for the investigator is to decide what constitutes a solvable technical glitch and what comprises a serious threat to the overall objectives. Experience is the best guide. If you have handled similar problems in the past, then you are the best judge. If you haven't, locate someone who has. In any case communicate your concerns to all involved parties as early as possible.

What If Things Go Better Than Expected?

How can you use good fortune to your best advantage? Most research triumphs are a blend of good times and bad. When good things happen during the course of your work, you may find yourself ahead of schedule or gaining confidence in the direction of your efforts. If you find yourself ahead of schedule, think ahead and use the extra time to stay ahead.

More often than not there will be subsequent phases of the work for which too little time has been allocated. Start the next step early or spend the time to address future problem areas of the plan. If the nature of the success you have achieved is to eliminate the necessity for some of the future work planned, you may be tempted to skip ahead. Such a change would constitute a significant departure from the original plan, so check with your superiors before proceeding on this altered course.

When Has the Project Been Completed?

A project will end when the basic objectives have been met. This view of the end is comforting in that you have specific objectives and a plan to achieve them, but disconcerting if the objectives change for reasons described above. If changes were controlled, discussed and documented throughout, endpoints should still be easy to identify. This is another reason why it is so important to establish a written consensus for each deviation in the plan.

Was the Project a Success?

If you stuck to your original plan and encountered no problems along the way, you were lucky. If problems required you to adapt your thinking, then real success was achieved. Remember, true failures are rare. The process of conducting research is one of constant evolution. If you have maintained an open mind and based your decisions on the facts uncovered by your work, your efforts were successful.

A Friendly Suggestion

If you are a new investigator or otherwise engaged in research that is new to you, take a lesson from the "old-timers." It's not that they have all the answers, it's just that they know how to ask better questions. They have had numerous opportunities to make their own mistakes, and if they have been successful, it is because they have learned from them.

THE RESEARCH

Are Bad Data a Myth?

Data are the medium of the scientific method, and can neither be good or bad. Data are the answers to the questions we pose, and it is the way we pose these questions that can be good or bad. Data could have intrinsic values: indeterminate, suggestive, or compelling in nature. Poorly posed questions often lead to indeterminate results, while exquisitely framed questions more often lead to compelling data. Therefore the secret to good research is in its design.

What Constitutes a Successful Outcome?

The answer to this question requires another: What are the specific objectives of your work? Must you produce a publication (basic research), a working model (industrial research), a reliable technique (applications research), or a prophetic example (intellectual property development)?

The specifications for success may vary significantly among these outcomes, so it might be worthwhile to verify your objectives with your supervisor or your collaborators.

What Source of Data Would Be Most Compelling?

If the answer isn't apparent, imagine yourself presenting data in front of a group of critical reviewers. What sort of questions or objections would you expect to hear? Answers to this question can be gleaned from seminars on topics similar to yours and from the scientific literature. The data published in peer-reviewed journals have stood up to the test of the review process and have been condensed to the most compelling evidence available to the author. You might also learn that the author applied an unexpected statistical analysis to support their conclusions.

Do You Have the Expertise to Obtain These Types of Data?

Do you have access to the specific equipment, materials, and methods necessary to perform your work? Finding access to one of these elements can provide access to the other, as can a network of friends and colleagues. Your desire for training might inspire someone to loan you the use of their equipment, along with their expertise.

What are your options if the equipment or expertise are unavailable to you? A review of the scientific literature might provide you with an alternative approach. For example, if tech-

nique *A* isn't available, the literature describing the development of that method will undoubtedly discuss techniques *B* and *C* and why they are inferior to technique *A*. Even if you have access to technique *A*, verifying your data via technique *B* or *C* might prove useful.

What Can You Do to Maximize the Reliability of Your Data?

Equipment and Reagents

Is your instrumentation working properly? When was it last checked for accuracy? An inaccurate spectrophotometer or pH meter could affect many aspects of your research. Do you possess all necessary reagents and have you proved their potency?

Have you considered your current and future sample needs? Will you employ statistical sampling in your experimental plans? You might save time, trouble, and money by analyzing your statistical sampling needs at the start of the project instead of returning to an earlier phase of the research to repeat a number of experiments. How will the data be collected, stored, and analyzed? How will statistics be applied, if at all?

Sample Issues

Replicates

A discussion about statistical analysis is beyond this book, but Motulsky (1995) provides practical guidance into the use of statistics in experimental design. Consider the use of statistics when determining the number of required replicates. Otherwise, you might find yourself returning to an earlier phase of your project just to repeat experiments for the purpose of statistical validation.

Quantity

How much material will you require over the short and long terms? Will the source of your material be available in the future, or is it rare and difficult to obtain? Will the physiological or chemical properties of the source change with time? What is the likelihood that the nature of your work will change, introducing new sample demands that require frequent sample preparations?

Should you prepare enough material in one episode to last the duration of your project? Sounds like a sure approach to minimize batch to batch variations, or is it? If the sample requirements make it practical to prepare an extraordinarily large amount of material, what do you know about the storage stability of the

prepared material? Will chemical stabilizers interfere with the research now or in the future? Periodic control assays of material stored over a long term might prove helpful.

If the sample is subject to minimal batch-to-batch variation during preparation, then multiple small samplings may be the most convenient approach, for this provides an additional benefit of providing fresh sample.

If you can verify or control for the long-term stability of your sample, large-scale sample preparations are usually preferred, since most samples reflect the state of their source at the time that they are obtained.

Quality

Generally speaking, samples of high purity require much more starting material, so one approach to controlling demand on sample quantities is to establish the requisite levels of purity for your application. Many assays and experiments have some degree of tolerance for impurities and will work well with samples that are only moderately pure. If you test the usefulness of different sample purities in your research, you might uncover opportunities to reduce the required amount of sample.

Are You on Schedule?

You will likely be asked for precise estimates of when you plan to complete your work, or for time points of certain research milestones. The answers to the previous questions should provide you with the big picture of the research and how the individual parts could affect one another. An accurate sense of the overall timing of the research ahead should follow.

This is also a good point to search your memory, or that of a colleague who has done similar work, to identify potential pitfalls. The goal is to eliminate surprises that tend to get you off schedule.

Which Variables Require Controls?

Consider the converse question: Which variables don't require controls? You might have to switch sample origins, reagents, reagent manufacturers, or instrumentation. As discussed in Chapter 2, "Getting What You Need from a Supplier," suppliers don't always notify the research community of every modification to a commercial product. Even control materials require their own controls. As mentioned above, you'll want to have proof that your large quantity of frozen control material is not degrading with

time. Considering the possible changes that can occur during the course of a research project, it's risky to conclude that there exists any variable that doesn't merit a carefully documented control.

The Roles of Reporting

When Should You Report Your Research Results?

In general, most project leaders and collaborators prefer to be kept informed, good news or bad. When your data are reproducible, discuss it with your research leader or senior colleague. These meetings also provide an opportunity to check that your colleagues' expectations for your research still coincide with your own.

If either party consistently appears surprised or misled, you might want to reevaluate the frequency and form of reporting. As discussed earlier, few research projects proceed exactly as planned, and these changes might require a change in the nature and scope of your reporting.

What Are Your Expectations When You Report Your Data?

Like most of life's endeavors, a research project begins with and is motivated by at least two very human desires. One desire is to uncover the truth no matter the outcome (the noblest case), while a second is to achieve our personal goals (the practical case). Research is not done in a vacuum and inherently contains biases. Consider these conscious and subconscious factors when you and your colleagues interpret data and offer conclusions.

Ideally there will be only one tenable interpretation of the data, but this is a rare outcome. Providing a fair treatment of the various interpretations in the report should lead to a dispassionate discussion that produces a consensus next step if not a conclusion.

What Are Your Options When Someone Attacks Your Data, Interpretations, or Conclusions?

The most common (and very human) initial response is to become defensive, to focus your energy on finding a weakness in your detractor's attack. A more productive route would be to welcome and embrace any contrary opinions. Pay attention to the details; make sure you thoroughly understand every aspect of their criticism. If you can objectively analyze your detractor's comments, the worst that can happen is for your research to be improved. One of the most productive phrases in the human vocabulary is the statement; "Maybe you're right, let's think

about it some more." Unfortunately, it is also one of the most underutilized.

THE REWARDS

Money is usually not the sole motivator. The practice of science requires much patience, a willingness to take risks, and the ability to wait months or years for the rewards. This requires a special kind of personal and professional commitment. Why did you choose to practice science in the first place? Curiosity and awe in the workings of nature? "Science in the service of knowledge and society" might elicit chuckles from some within and outside the scientific community, and that's a shame.

Hopefully you will find ways to enjoy the scientific process on a daily level, working to achieve the big things while relishing small accomplishments. Consider the benefit of recognizing and rewarding the achievements of others and you, and by all means, have fun along the way.

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Getting What You Need from a Supplier

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HOW CAN YOU WORK MOST EFFICIENTLY WITH YOUR SUPPLIER?

Companies hire researchers, license ideas, generate much useful data that aren't always published, and fund scholarships. Familiarity with the corporate mindset, structure and resources can help you obtain what you need and avoid problems you don't want.

All Companies Are the Same?

All companies are not the same, and this fact is becoming truer everyday. Today a company selling research reagents may consist of a scientist turned entrepreneur working out of a home office. From a home in the midwest, the scientist might incorporate in Delaware. Once set up as a corporation, she may find someone else to make the wonder reagent in California and then arrange for some other company to package, label, and distribute the wonder reagent. No board rooms, no business lunches, and practically no one for a customer to complain to when things go wrong.

At the other end of the spectrum is a corporation doing business in 50 countries with sales in the hundreds of millions of dollars. Of course, with a well-known name on the tip of every scientist's tongue and a great reputation, super big company is much easier to find and much easier to reach for help you need. Don't count on it.

Each company has its own goals, dreams (i.e., visions) and personality. Within large companies, each division might have a distinct philosophy and operating strategy. Satisfaction with the products and services from an instrument division doesn't guarantee similar performance from a reagent division.

Big Is Better, Small Is Better?

Whether Big is better or Small is better depends on whether they fulfill your needs. Small will often have the greater desire, since even the smallest amount of business you send to them will be significant to Small's bottom line. But it will often lack the resources, knowledge, or external contacts to fulfill your needs that are out of the mainstream of its operation. This conflict may result in Small promising you something it can't deliver.

Big on the other hand will tend to have access to more internal and external resources. A special request may be easily within Big's knowledge and capacity to deliver. But how much are you willing to buy? If it isn't enough, Big won't have the incentive to do something unique for you. It just wouldn't make economic sense. If Big does its job right, you will quickly know it isn't willing to deliver, and you can go looking for another supplier.

Is the Product Manufactured by the Company That Sells It?

Some companies only sell products which they conceive, develop, and manufacture. Other suppliers only distribute products manufactured by other firms. Many, perhaps most companies, do some of both. The true manufacturer of a product may not be indicated on a package. If you are satisfied with the product's performance and support, its origin isn't an issue. But it may become an issue when problems arise, since the original manufacturer will generally have the most knowledge about the product.

Does a Company Test Every Application for a Product?

The research community regularly generates novel applications for commercial products. Combine this with limited application resources by suppliers, and the result is that a company tests only those applications it judges most important to the majority of the research community. If your application isn't mentioned by the manufacturer, odds are that application hasn't been tested or has been attempted an insignificant number of times.

It never hurts to contact the company. While the company may not have tested the product in your particular application, your call might persuade the company to do so. It is not uncommon for suppliers to provide product at little or no cost in exchange for application data generated by the customer. Manufacturers also might have a database of researchers who've attempted your application. The *Methods and Reagents* bulletin board located in the Biosci Web site (*http://www.bio.net/hypermail/methods/*) is a productive location to ask if a product has ever been tested in your particular application. This site can also help you locate and obtain hard-to-find reagents.

How Well Will the Product Perform?

As alluded to throughout this chapter, it is impossible for a supplier to guarantee the performance of every product with every sample source. But in today's competitive marketplace any reputable supplier will do its best to guarantee that advertising

claims match actual performance. In addition third-party reviewers help ensure advertising claims aren't overly exaggerated. These third party (and hopefully objective) reviews of commercial products are provided at the following Web sites:

- The Scientist, *http://www.thescientist.com*
- BIOSCI Methods Group, *http://www.bio.net/hypermail/methods*
- Biowire, *http://www.biowire.com*
- Biocompare, *http://www.biocompare.com*

Are Identical Products Manufactured Identically?

When different companies seem to manufacture identical items, there may be differences in the production methods. For example, company *A* might quantitate the activity of *Taq* DNA polymerase after packaging because company *A*'s automated dispensing equipment might cause foaming of the protein and thus instability. Alternatively, company *B* may never test the activity of the *Taq* polymerase after packaging because it is manually dispensed, a procedure that doesn't harm the activity of the enzyme. The difficulty for you is that switching manufacturers may change performance more than you expect.

Will a Company Inform You When They Change the Product?

Manufacturers prefer not to change production strategies, but sometimes no choice exists: raw materials become unavailable, broken equipment can't be replaced, or people leave the company and take away the knowledge for synthesizing a product.

Changes are not always announced to the public. Responsible companies try to judge the impact of a change and determine its effect on the research community, but it is impossible to correctly predict the impact for everyone. If the change is thought to be significant, products might be labeled *New and Improved*, instructions might be changed, or packaging might be changed. If you're not sure if the changes will affect your research, contact the company and get the details of the modifications. The manufacturer might have experimental data that will help you evaluate their impact.

HOW CAN YOU WORK MOST EFFICIENTLY—AND PLEASANTLY—WITH A SALES REPRESENTATIVE?

The preceding section discussed the inner workings of equipment and reagent manufacturers. The next discussion focuses on

strategies to manage your relationship with a company's sales representative (sales rep).

What Can a Sales Rep Do for You?

A good sales rep can help you determine what you need, what you don't need, and the most cost-effective way to get it. As a conduit to a company's administrative and scientific resources, a sales rep can help you resolve bureaucratic problems, receive technical information in a timely fashion, make sure you clearly understand all the nuances of a price quote, and help you obtain special order items.

What Should You Expect from a Sales Rep?

While you and your sales rep may think differently, you should be made to feel confident that advancing your research is important to your sales rep. Respect for you and your time, and the confidentiality of your research should also be maintained. As discussed below, good salespeople love to know "the inside scoop" and take personal pride in their customer's research, but you shouldn't have to worry that their exuberance for your work results in confidential details discussed with your competitors. The best way to determine a rep's trustworthiness is to discuss other work in the field. If you're suddenly learning details about the competition that you would never share with the outside world until papers are published, you have reason to wonder if your ideas are being similarly discussed. Discussions about what someone else is buying (unless the researcher has agreed to serve as a reference) also is cause for concern. You have every right to expect that even your most mundane dealings with the company are kept confidential.

Is it reasonable to expect your rep to be thoroughly familiar with the technical aspects of their products? If they represent a catalog of 13,000 items, probably not. If the product line is more limited and highly technical in nature, you should expect a high degree of technical competence. In either case a good rep employed by a company that truly cares about their customers should be able to deliver answers to any questions within two to three business days.

As is true with business in general, your sales representative is probably managing her territory by the Pareto principle. That is, 80% of her business comes from 20% of the customers. While the majority of reps want desperately to assist all customers and treat them equally, the reality is that the elite 20% are going to get the

lion's share of her attention. This is simple survival, as losing all or part of the business at those key accounts is likely to cost her significant commissions, and quite possibly her job. This doesn't mean that you should ever feel like one of "the-less-than-elite" 80%. You should always feel like the only person in the world when working with a sales representative, and a cell phone ringing in a briefcase is not something you should have to deal with.

How Can You Get What You Want from a Sales Rep?

Understand Their Motivation

A sales rep has at his core a rational self-interest. That is, he must do the things that will benefit his performance and ensure survival. While some reps are self-centered, others recognize the interdependent relationship he has with his customers. Your success is his success, though the converse is not true.

Companies typically motivate their representatives through sales contests and commission structures. Top salespeople often receive paid vacations, and commissions are often structured to move certain product lines. It is true that sales positions are some of the best paid positions in a company, and most sales people are to some extent money-motivated. But you still have every right to expect that products are being offered to you because they will solve your problem and not because they will make your rep the most money.

The best salespeople truly enjoy helping others. They enjoy the bonds that are established, and revel in the feeling that they are "on the inside" regarding research. At their heart, many salespeople also have a "need to please," and they receive a real boost when they've done something for you and you've noticed. If you have criticism, also feel free to relate it, and express your expectation that something be done to improve the situation. While a poor rep may avoid you once you've complained, the good ones will recognize your comments as an opportunity to change your opinion of them, their company, and therefore create a satisfied customer that will likely buy more product. The need to please can be a great motivational tool to get what you need from your sales representative.

Manage the Relationship

Evaluating what you need from the company, and how you want those needs managed will maximize value from the relationship. Your sales rep doesn't know what to expect because every customer is different. He deals with multiple people at each account:

the researcher, purchasing, receiving, safety, and so on. The relationship with your sales representative is a lot like dating; it can be ruined by unexpressed expectations. For that reason it is imperative that you express exactly what you need from this person. Do you need to see her every week? Do you want to be on the top of the list for trying new products? Do you simply want to see them on your terms, that is, "don't call me, I'll call you?" There is nothing wrong with expressing your wants. Rather, you are giving direction to someone in desperate need of it.

A good sales representative keeps a profile on important customers. Items in that profile may include area of research, money available, general temperment, and if you tell him, exactly how you like to be handled. Your rep will appreciate this, since it provides him a chance to better manage *your* expectations. There may be ground rules he can't accept, such as a weekly visit. He may have distant accounts that will demand his time. Perhaps you can compromise on an email inquiry along with a bi-weekly visit. Don't wait to discuss your needs; tell him on his first visit, reach compromises if necessary, and start working together.

You'd never dream of running an experiment without proper controls and measurements, so why treat this vital relationship any differently? If you've laid out your expectations, you now have the means to evaluate your sales rep. Exceptional sales representatives will automatically measure themselves against your expressed wishes. Feel free to ask for evidence when you review the relationship. Good sales reps will have an answer ready.

You should expect to review the relationship at some regular interval. Perhaps your needs have changed, or you've noticed some slippage or improvement in the performance of your rep. Don't hesitate to ask for a quick meeting to reassess.

A sales rep can enhance the relationship if she helps you manage your expectations of the company. She may ask you to forecast repeat usage or estimate future needs as a way to give you current information on availability and delivery. If you need one liter of a reagent, and that volume represents three months' production for that company, your rep must help you manage your expectation for immediate delivery.

Leverage

Serving as a reference is a great way to gain influence with your sales rep. There is no sales tool more powerful than a satisfied customer. If you're happy with your representative, her product, and the company, offer to serve as a reference. Your sales rep will be delighted, and this could help get you preferred treatment. Don't

hesitate to explain why you're making this offer, and what you expect in return. This is part of "negotiating the relationship," and you don't want to make such a generous offer without expecting something in return.

ORDERING A CUSTOM PRODUCT

A product whose composition or quantity differs from the catalog item may be considered custom by many manufacturers. Such specialized items tend to be expensive; the following suggestions are provided to help you obtain the desired item at minimal cost and aggravation.

Know Exactly What You Need

Vague specifications cause problems. If you call a company and ask for 100 liters of phosphate buffer at pH 7.5, will it matter how the pH is adjusted? Does it matter whether sodium or potassium phosphate is employed?

Complete and detailed communication with the manufacturer is crucial. You as the buyer must take charge to ensure that the company tells you what information must be provided, specifications, and all other details. Ideally a supplier will ask several detailed, and maybe obvious, questions in order to truly understand your needs. Be suspicious of companies that ask little and promise everything. Some custom products are simple to specify, but it might not be feasible to thoroughly describe complex, or novel, products. In these cases it may be helpful to describe to the manufacturer what you don't want as well as what you do.

Know Your Quantity Needs and Frequency of Delivery

Manufacturers can't determine cost, nor their ability to deliver the proposed product, without knowing accurate quantity requirements and the frequency of orders.

Know Your Spending Limits

Although you do not want to negotiate price immediately with a manufacturer, you should know what you are willing to pay for the custom product. This will shorten your list of prospective manufacturers.

Document Your Needs

A thorough, comprehensive record of your answers to the preceding questions will prove invaluable during your conversations with suppliers.

Identify the Right Manufacturer

Determining which suppliers truly want your business is not a perfect science. Some manufacturers will tell you when they can't fulfill your needs, while others will hint but won't say no outright. Hints can include requests to buy a very large minimum quantity, suggestions of alternate products, or the news that delivery is not possible for ages. The trick is that these comments aren't always hints to chase away your business, but legitimate technical or business concerns that can't be avoided. After dealing with two or three potential suppliers, you will be able to identify those suppliers who are serious about your business.

Obtain a Document That Details the Order Acknowledgment

Require the company to document, in excruciating detail, what they will produce for you. If the description isn't complete, detailed, and accurate, make the company do it again. If something goes wrong, it will be your only proof of what the supplier promised to do for you.

RESOLVING PROBLEMS

There is nothing inherently negative in the word "problem." Its origin lay in phrases meaning "anything thrown forward" or "to lay before." A problem provides opportunities to sharpen your research skills and ultimately improve the reliability of your data. Keeping an open mind and an inquisitive nature when problems arise will minimize your frustration and speed the problem's resolution.

Problem prevention is faster than problem solving. To prevent problems from occurring, read the information supplied with the product. Suppliers usually work hard to determine what information is required to successfully use a product. Although reading directions may be boring, it can prevent many problems.

If you wish to use a product in a way that is not clearly described by the directions, consider asking the supplier the following questions before proceeding with the experiment:

- Has the product been successfully applied to your intended application? Even when the answer is no, suppliers could help you determine the likelihood of success.
- Is it safe to modify the procedure in the manual? Changing the volumes of reagents, incubation times, sample preparation, temperature of reactions, or any number of other seemingly minor

changes to the procedure may have large effects on the results obtained. Before deviating from any of the directions supplied with a product, it is best to call the manufacturer and see if they have any information on the effects of making that change in the procedure.

- Can the storage conditions be modified? Storage temperatures other than what the supplier recommends may compromise the stability of the product. This is especially likely if the product undergoes a phase change when stored at an alternate temperature. In addition, and maybe more important, products may become hazardous when stored at the wrong temperature.

Solving Problems by Yourself

In a perfect world, six steps will solve any problem:

1. Define the problem. What do you see?

The first step in any problem-solving activity is to fully understand the nature of the problem without drawing conclusions. Understanding a problem consists of describing all the factual aspects about the problem. Do not try to determine what caused the problem. That step comes later in the process. If a PCR reaction failed to give a product, the problem description is simply that no product was obtained. You may have used a new brand of *Taq* DNA polymerase in the reaction, and this is an important fact to state. But it is not a good idea to immediately draw the conclusion that the correlation of the new brand of *Taq* polymerase and the lack of a product means that the enzyme is bad. Rather, a more thorough analysis of all of the parameters involved should be done. Did the reaction buffer change? Did the thermocycler function properly? Was the template DNA the same as previous reactions that worked? Was a different method of DNA preparation used? And so on. Once the problem is fully described with all reasonable parameters understood, then some simple, obvious causes can be ruled out.

2. List *all* the theoretical explanations that could cause the problem reported in step 1, including the obvious ones.

The majority of problems stem from the most likely causes. Before searching for the esoteric sources, rule out the most likely explanations. This step of troubleshooting is often aided by asking another person for some help. An outside party will have a different perspective on the situation and may think of an obvious cause that escapes you. Obvious causes of a problem are always hardest for the person closest to the problem to see. For example, is a piece of equipment plugged in? This is so obvious that it is an

often overlooked source of equipment problems. In the *Taq* polymerase example above, the equivalent question asks if enzyme was added to the reaction. Both are very likely simple errors that can lead to endless hours of troubleshooting until they get accidentally stumbled over.

3. Gather all the data that you have regarding the problem.

Was the control tested?

Instruments are often supplied with a standard for verifying the operation of the equipment. Analogously, reagents kits are often supplied with a control sample. If you have never used an instrument or a kit previously, consider testing the standard or control supplied before proceeding with any experiments.

Standards and controls are also extremely valuable when things go wrong. If the standard or control was not used and a problem appears, the first experiment to do is to test the standard or control. Changing experimental variables will be a complete waste of time if an instrument is out of calibration or the kit has deteriorated in some way. In addition, if you ask the supplier for help, one of the first questions that the supplier of the instrument or the reagents will ask is whether the standard was tested or whether the control in the kit was used. If your answer is no, it is very likely that you will be asked to test the standard or control and then call back. The reason for the question is that the supplier is trying to determine whether their product is the cause of your headaches or whether some other experimental variable is the problem.

How long was the product stored and under what conditions?

Properly maintained, common laboratory instruments do not deteriorate over time during storage in dry conditions if protected from dust. Instruments need routine maintenance and regular calibration, but aging is not a typical problem.

On the other hand, many chemicals and biochemicals do deteriorate over time. This deterioration is often accelerated by improper storage conditions. Before using any chemicals or biochemicals, verify that the chemical has been stored under recommended conditions. If it has not, either do not use it or call the supplier to see if they have information on the effect of alternate storage conditions.

Even if the chemical/biochemical has been kept at proper storage conditions, it is a good idea to determine the approximate age of the chemical or biochemical. It is risky to use a reagent whose age can't be determined.

Manufacturers may or may not have expiration dates on their chemicals. If they don't, the manufacturer should still be able to tell you, when given the lot number, when the product was made, and some estimate for how long a chemical can be safely stored under recommended conditions.

When asking about expected shelf life, have a clear idea of what you really need to know and why you are asking. Many manufacturers have never performed formal stability tests on their products and therefore can only give you anecdotal information from their experience. In many cases this will be sufficient.

If there is a chance that your research may lead to a commercial product that will be regulated through cGMP (Federal Register 21 CFR parts 210, 211, and 820) regulations, determine if the information the supplier has will be sufficient for your needs. Also be aware that the manufacturers will only be able to give you information about their product, in their packaging, under their recommended storage conditions. If you take that chemical and prepare a buffer or any other type of formulation with the chemical, their information cannot be extrapolated to your use of the chemical, and you will need to be responsible for the stability data on your formulations.

For this very reason, if you ask a manufacturer for the storage stability of their reagent once it has been applied to a procedure, they are likely to respond to you that they don't know. This answer is not to be difficult but is to prevent giving misleading information. You are likely to get more useful information from the manufacturer if you explain why you need the information. The manufacturer will then be able to give you a more complete and useful answer.

Getting an expiration date from a manufacturer is only as helpful as knowing exactly how that date was derived and knowing what it means. Products that pass their expiration dates may very well be sufficiently active for your purposes, since the date may be very conservative. If you are performing noncritical work, it may be acceptable to use chemicals past their expiration dates, once you know how a manufacturer determined the expiration date applied to the package.

Was the procedure modified?

If you deviated from the manufacturer's instructions, be sure to be able to exactly describe all changes. Even the slightest deviation may lead to suboptimal results.

4. Eliminate explanations from step 2 based on the data described in step 3.

5. Design and execute experiments that address the remaining explanations.

6. Eliminate the remaining explanations from step 2 based on the new data generated from the experiments of step 5.

Example of Using the Six Problem-Solving Steps: The DNA That Wasn't There

Step 1. Define the problem. What do you see?

One hundred ng (as quantitated by spectrophotmetry) of a 500 bp DNA fragment were loaded onto a 1.0% agarose gel and electrophoresed under standard conditions; ethidium bromide staining revealed the marker bands, but not the 500 bp fragment of interest. No staining was observed in the wells. Also two different DNA markers ranging from 1000 to 100 bp were loaded on the gel and ran as expected.

Step 2. List all the theoretical explanations that could cause the observations in step 1, and don't forget the obvious ones. Don't worry about the feasibility of your explanations yet.

a. The DNA was destroyed by a nuclease contaminant.
b. The DNA never migrated away from the loading well.
c. The DNA ran off the gel.
d. DNA was never present in the loaded sample.

Step 3. What data do you have?

a. Two lanes with different DNA markers appeared as expected.
b. The same gel box, power supply, and ethidium bromide used in your work successfully visualized DNA before and after your experiment.
c. Your spectrophotometer correctly quantitated a series of DNA standards in a concentration range similar to your 500 bp sample.

Step 4. Eliminate explanations from step 2 based on the data described in step 3.

a. *The DNA was destroyed by a nuclease contaminant.*

Then why weren't the two different markers similarly digested?

b. *The DNA never migrated away from the loading well.*

Not likely. Ethidium bromide staining did not appear at the wells.

c. *The DNA ran off the gel.*
Some of the markers were smaller than 500 bp, and they didn't run off the gel.

d. *DNA was never present in the loaded sample.*
Spectrophotometer data suggest that DNA was present. The same spectrophotometer accurately calculated the concentration of other DNA samples.

Step 5. Design and execute experiments that address the remaining explanations.

At face value all the possible experimental explanations have been eliminated. Or have they? Perhaps we should take a closer look at the spectrophotometer data.

The spectrophotometer used in the experiment was programmed to report the concentration of the samples in micrograms per milliliter.

Concentration (μg/ml) 500 bp Fragment	
Reading 1	40
Reading 2	35
Reading 3	40

The data look reasonable and reproducible, but just to be thorough, let's look at the absorbance values at 260 nm from these readings.

Absorbance at 260 nm 500 bp Fragment	
Reading 1	0.008
Reading 2	0.007
Reading 3	0.008

Concentration calculation:

$$0.008 A_{260} \times 50\,\mu\text{g/ml} \times 100 \text{ (dilution factor of sample)} = 40\,\mu\text{g/ml}$$

The samples were very dilute, outside the preferred range for correlating absorbance with concentration and possibly beyond the sensitivity of the spectrophotometer, as discussed in

Chapter 4, "How To Properly Use and Maintain Laboratory Equipment." Furthermore this sample was a 1:100 dilution of the stock material, increasing concern that the sample was too dilute for accurate quantitation.

Step 6. Eliminate the remaining explanations from step 2 based on data generated from the experiments of step 5.

Measure the absorbance at 260 nm of a 1:10 and 1:100 dilution of the DNA sample.

Absorbance at 260 nm

	1:10	1:100
Reading 1	0.006	0.008
Reading 2	0.008	0.007
Reading 3	0.009	0.008

The experiment generated nearly identical absorbance values for both dilutions, implying that the samples are below the sensitivity of the spectrophotometer. Repeat the absorbance measurements of the undiluted stock to determine an accurate concentration.

Solving Problems with the Help of the Supplier

Gather All Pertinent Product Information

Once you determine that the control or standard has failed, the product is not extremely old and you didn't modify procedures from those recommended by the manufacturer, it is time to start thinking about calling the supplier. But before picking up the phone, gather all the information that you will need. The supplier will want the product number and the batch or serial number.

If it is an instrument, the supplier will usually ask for the serial number. Ideally this number is best recorded when the equipment is first received. Once an instrument is installed, it may be practically impossible to get to the number because of the inaccessible place the manufacturer chose to put it.

Reagents do not typically have a serial number but will often have a lot or batch number. This number is key to the supplier because it will give them the information that they need to be able to determine when the product was made and to trace back to the original manufacturing records. These records will help the manufacturer determine whether anything unusual happened during the manufacture of the product that might be causing your problems.

Are Comparisons Truly Side-by-Side?

If you are planning to describe to the supplier comparison experiments you did to troubleshoot the problem, be prepared to describe the exact conditions of the experiment. The supplier will want to know whether any comparisons performed were truly side-by-side. A true side-by-side comparison is one in which all variables are identical except for one. For example, a problem might be that a first-strand cDNA synthesis reaction failed to yield first-strand cDNA after changing to a new vial of reverse transcriptase. On the surface it may seem that the two reactions are side-by-side. But, if the mRNA applied in the reaction is from a different preparation than the mRNA used in the successful reaction, then two variables are different—different vials of reverse transcriptase and different mRNA samples. If the mRNA was degraded in the second sample, this would cause the first-strand reaction to fail and make it appear as if the reverse transcriptase is at fault. Being able to accurately describe how similar comparisons truly are will speed the problem's resolution.

Contacting the Supplier

Who to Call?

When calling a company for help, don't have a preconceived idea on who you should be speaking with. Some companies may have you deal with research and development scientists, others with full time technical support people, and some may first have you deal with your salesperson before passing you on. Don't assume one way is better than another. Each method has its positives and negatives, and each when successfully implemented by the company should be able to get you the help you need. Asking a company to follow the method that you think is best may cause several problems for the company including lack of documentation of your call, inability to authorize credit if required, and general confusion by the person who initially handles your call.

Record All the Details of the Conversation

You will want to write down all the basic information about each person you deal with, including the person's full name, their department, and the date and time of your call. If the situation continues over several days or weeks, what seems like basic facts you can't possibly forget will start to blur. Keeping an accurate log of each contact will also increase your credibility with the company, a benefit if you ultimately need to pursue the issue with supervisors and managers.

Finally, recording the name of the department will be useful when trying to contact the same person in the future. Even small companies may have two people working who have very similar or identical names and the department name will help locate the correct "John Smith." In large companies, service calls may be routed to various parts of the country, and it will be impossible to contact the same person in the future without knowing the department or even the city where the representative works.

State the Problem, Not the Conclusions

Describing the facts of the problem and not stating your theories on the underlying cause has several benefits. First, it gets you an unbiased opinion from your supplier's representative. If you give your ideas on the underlying cause and the person agrees with you, you may not have gotten the person's best judgment of the situation. Second, calling a representative and stating that you know there is a problem with their product can make some people very defensive and uncooperative. This may result in both parties being angry. The company may lose a customer, but your problem won't be any closer to being resolved. Finally, by stating just the facts, it will help you keep an open mind to the information that the company representative is telling you.

Ask If Anything Has Changed with the Product

If you are experiencing a problem with a product with which you have a history of success, it is useful to investigate whether anything has changed with the product. If you ask the representative whether anything has changed with the product and the person quickly says no, follow up the question with a list of specific items. Ask whether raw materials, equipment used in manufacturing, product specifications, or employees making the product have changed. The point of being specific in asking what may have been modified is that the person on the phone may not consider the wide range of alterations that could affect product performance. By specifically listing various potential changes, you are more likely to get the person to fully investigate whether everything is identical about the product since the last time you bought it.

Let People Call You Back

Good answers to your questions often require further investigation by several parties. Your question is likely just one of 20 to 50 handled that day by the representative. The person might have

to check records, speak with people having specific knowledge, or just quietly analyze what you have said and consider possible causes. Whatever the reason, it is to your advantage to let the person take the time and investigate further before calling you back.

Remember to Thank the Person

As obvious or silly as it may seem, thank the person who has been helping you on the phone. Even helpful people usually try a little harder to assist those who treat them well.

If the person is unhelpful or obnoxious, keeping a polite, professional approach will increase your credibility with company superiors who later get involved with the problem. Losing your cool will only make management feel that their employee was abused.

If You're Still Unhappy

Even after trying to get a problem resolved with the company, you may still be very unhappy with the results. You might not have been treated fairly, or perhaps your expectations about what the company could do for you were too high.

What Is Reasonable to Expect?

Generally, it is only reasonable to expect the company to reimburse you for the product purchased. A statement indicating this is typically included in catalogs and is often present in the invoice that arrived with your order. The statement will exclude liability for your time, other products you may have used, lost research time, or other real costs you incurred due to a product that failed. Expecting reimbursement for any of these items is very unlikely, even if the company finds it was at fault for causing your headaches. You may be able to negotiate more than the replacement cost of the product you bought, but it will definitively require negotiation.

Who to Complain To?

Often the representatives who work directly with customers have very little freedom in what they can do to satisfy a customer. If you request reimbursement or assistance beyond what is typical, you will need to work your way up the corporate hierarchy. (Also it never hurts to contact your sales representative in these situations; they might be anxious to serve as your advocate.) Ask to speak with a supervisor. If they don't directly solve your problem,

they can usually help you find the appropriate people. In some cases, calling the president or the person responsible for the manufacturing site may get the best response. It will just take patience working up the corporate ladder until you find someone who has the authority and resources to give help beyond the ordinary.*

**Editor's note: Yelling rings most effectively in the ears of upper management, not low-level personnel.*