[1] Equipping and Organizing Comparative Molecular Genetics Laboratories

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Introduction

Rapid advances in biochemical and molecular genetic technology are revolutionizing many fields of biological inquiry. Nowhere is the effect of these new tools more keenly felt than in evolutionary biology.[1] For here, the new technology is not merely changing the course of research within a discipline; it is merging what were previously unrelated fields. Thus, modern systematists must now concern themselves with such unlikely topics as protein structure and function, while molecular geneticists must consider their data in the light of evolutionary theory. This melding process can be especially difficult for biologists without extensive training in biochemistry or molecular genetics who nonetheless find molecular data sufficiently compelling to try to use the techniques themselves.

Our purpose in this chapter is to give guidance to the newcomer on how to organize and equip a laboratory effectively for comparative molecular research. General articles on laboratory setup can be found in molecular methodology publications.[2,3] In molecular evolutionary studies, however, the focus on comparative analysis dictates special emphasis on streamlining repetitive procedures, adapting techniques for a variety of organisms, and managing many samples efficiently. We shall endeavor to orient researchers to the general considerations involved in planning a laboratory for molecular evolutionary studies. We briefly describe the techniques available and criteria for choosing among them. We suggest rationales for selecting equipment and strategies for the physical organization of the laboratory. Throughout, we highlight possible problems and solutions.

Setting Goals

Starting a molecular laboratory requires both consideration of the ultimate questions being addressed by the research and evaluation of the

[1] M. Clegg, J. Felsenstein, W. Fitch, M. Goodman, D. Hillis, M. Riley, F. Ruddle, D. Sankoff, P. Arzberger, M. Courtney, P. Harriman, C. Lynch, J. Plesset, M. Weiss, and T. Yates, *Mol. Phylogenet. Evol.* **1**, 84 (1992).

[2] D. D. Blumberg, this series, Vol. 152, p. 3.

[3] C. Orrego, *in* "PCR Protocols" (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.), p. 447. Academic Press, New York, 1990.

current research environment available to the researcher. The rapid proliferation and simplification of molecular techniques presents investigators with what at times may seem a bewildering array of possible approaches to problems. Yet, from the viewpoint of many evolutionary biologists, these procedures are still expensive, laborious, and time-consuming. Thus, it is important at the outset to assess the resources and support available for the contemplated research, and to pay attention to choosing techniques that are well suited to the problems at hand.

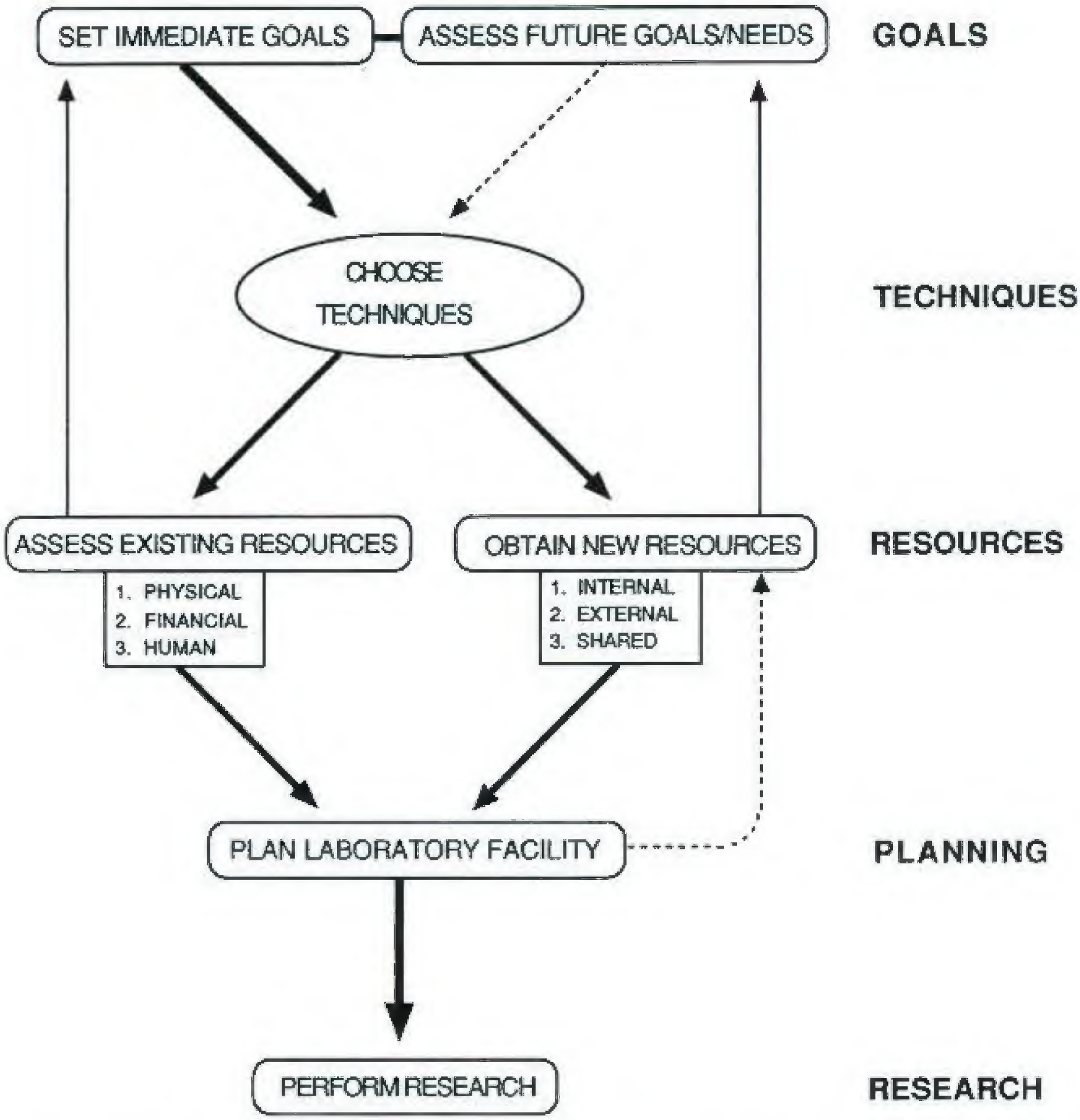

FIG. 1. Flowchart for laboratory planning. The five major stages in planning a laboratory facility are listed to the right. Arrows in the chart indicate the direction of progress through stages and how information gleaned at one stage can cause reconsideration of decisions made at a previous stage.

The following issues will merit consideration: (1) What frequency and intensity of laboratory work is expected? Some scientists need to obtain molecular data on one or a few specific questions; for others it will be an ongoing focus of research. (2) What technique or techniques will be used? Success often hinges on choosing a technique well suited to the research problem. The scope of the laboratory operation, in turn, will increase with the number and complexity of techniques to be implemented. (3) What resources already exist? What resources are obtainable? Given the cost and complexity of molecular research, it is crucial to assess the physical, technical, and human resources of the home institution and the local environment, and to be realistic about opportunities for obtaining new resources. (4) Can resources be shared? Virtually all molecular investigators share some laboratory resources. The extent of sharing may range from a few pieces of large equipment in a traditional department comprising many single-investigator laboratories to sharing of a single, centralized molecular facility among several investigators. Collaboration among research groups can provide the means to address scientific questions without setting up multiple independent research units.

Careful consideration of these basic issues at the outset will lead to more effective planning and a cost-efficient facility better designed to meet the needs of the individual and the institution. A flowchart (Fig. 1) helps illustrate the interrelationships of these issues and the feedback effects they may have on one another.

Choosing Techniques

The physical organization of a laboratory will be dictated by the techniques to be implemented there. Thus, it is essential to decide what techniques will be used immediately and what techniques might be attempted at a later date. Six major comparative molecular techniques are in common use today in evolutionary research. These are isozyme electrophoresis, comparative immunological methods [especially microcomplement fixation (MC'F)], DNA–DNA hybridization, restriction enzyme analysis, random amplification of polymorphic DNA (RAPD), and DNA sequencing. Two other methods, molecular cloning and the polymerase chain reaction (PCR), are not inherently comparative, but they provide the foundation on which a number of DNA techniques are based. We first consider the major factors involved in choosing among techniques, then discuss the advantages and disadvantages of each. Table I summarizes information presented below that will be helpful in selecting techniques.

TABLE I
CONSIDERATIONS FOR CHOOSING TECHNIQUES[a]

Technique	Optimal divergence range	Complexity	Cost	Time and labor	Nature of samples	Comparabilit
Isozyme electrophoresis	Lo/Med	Lo	Lo	Lo	Multiple tissues	Good
Microcomplement fixation	Med	Hi	Med	Med/Hi	Purified protein	Fair
DNA–DNA hybridization	Med	Hi	Med	Med/Hi	Large quantities of DNA	Fair
Restriction enzyme analysis	Lo	Med	Hi	Med/Hi	HMW DNA	Good
DNA sequencing	Lo/Med/Hi	Hi	Hi	Hi	Any purified DNA	Excellent
Random amplification of polymorphic DNA	Lo	Med	Med	Lo	HMW DNA	Fair
Polymerase chain reaction	N.A.	Med	Med	Lo	Any purified DNA	N.A.
Molecular cloning	N.A.	Hi	Hi	Hi	Any purified DNA	N.A.

[a] N.A., Not applicable, since technique is not a comparative method, but forms the basis for othe
HMW, high molecular weight; Lo, low; Med, medium; Hi, high.

Matching Resolving Power of Techniques to Genetic Divergence of Organisms

Most research problems in molecular evolutionary genetics ultimately involve questions of relationship. However, the degree of relationship may vary dramatically, from comparisons of related individuals within a population to comparisons of taxa that diverged early in the history of life on Earth. There is a general correlation between degree of relationship among organisms and the level of genetic similarity among their genomes. Again, the level of similarity can vary widely: the genomes of clonally reproducing organisms may be nearly 100% identical, whereas those of distantly divergent taxa may retain sequence similarity only in a few, highly conserved genes. The level of genetic similarity among the genes or genomes to be compared is the primary determinant of the applicability of the various comparative molecular techniques. Each technique is best suited to a particular range of divergences among the organisms being compared. Each technique probes genetic questions at different levels of resolution, from direct DNA sequence comparisons to inferred amino acid sequence variation. Some techniques, such as DNA sequencing, can be adapted to a broad range of divergences; others are not so versatile. Failure to appreciate

the relationship between gene or genome divergence and resolving power of a technique can lead to a data set that is largely irrelevant to the original research focus.

Complexity

To use some methods successfully requires more background and experience in biochemistry than others. Most comparative techniques involve repetitive procedures that seem deceptively simple to learn when all is working well. When a technique fails to work properly, however, considerable expertise may be required to discover the problem and get the research back on track.

Cost

It is convenient to think of costs as falling into three categories, namely, overhead costs of setting up and maintaining the laboratory, consumables cost of the supplies actually used up in a particular project, and personnel costs. At present, initial overhead costs might range from $20,000 or less for a laboratory concentrating on isozyme surveys to several hundred thousand dollars for a laboratory fully equipped to implement a wide range of techniques. Setup costs will vary dramatically depending on preexisting resources and physical location, so it is important to explore the local environment of the proposed laboratory (see section on assessing environment and resources below). The annual cost of consumable supplies will range from a few thousand dollars per active researcher for isozyme work to $10,000–$15,000 per person for the more expensive techniques. At many university laboratories, personnel costs may not be a major factor if salaries and stipends come from sources other than research funds. At other institutions, however, where all expenditures come from a common pool, personnel costs are likely to be an important budget item.

Time and Labor

The major comparative techniques differ substantially in the amount of time and effort they require. Some labor-intensive techniques are more appropriate when relatively small numbers of comparisons are to be made; other techniques can be readily applied to large numbers of samples.

Nature of Organism and/or Samples

The techniques differ in the amount and type of sample required. The physical size of the organism may limit the amount of tissue that can be obtained from each individual, and may make some techniques difficult or

impossible to use. Some techniques work best with certain kinds of tissue. Other techniques (e.g., isozyme electrophoresis) benefit from the availability of several tissue types. Most techniques work best with fresh or frozen material, but some can be used with samples that are thousands of years old.

Comparability across Studies

The ease with which a data set can be compared to other studies is an important consideration. DNA sequencing provides the greatest advantages in this regard. Because the method involves the absolute determination of nucleotide sequence, the data can be compared to other sequences determined completely independently. Some data derived from other techniques can be compared in a general way (e.g., isozyme genetic distance, sequence divergence estimated from restriction enzyme analysis), whereas other comparisons are best made side by side in the same laboratory (e.g., establishing homology of isozyme alleles or restriction fragment bands).

Techniques: Pros and Cons

Isozyme Electrophoresis

The examination of proteins by electrophoresis has been the single most popular technique in molecular evolutionary genetics. It can provide useful information for studies at the population and species level.[4-6] For some taxa where genetic distances tend to be low, such as birds, the useful range of isozyme electrophoresis extends to the genus and even family level. It is the least expensive technique, both in terms of overhead and cost of consumables. Large sample sizes can be handled readily, and the technical demands are relatively minimal. Electrophoresis and staining conditions are often similar throughout major groups of organisms, although some optimization is required for most studies.[4-7] In addition, gene expression varies by tissue, so having a variety of tissue types available for study increases the number of genes that can be assayed. Although data from separate studies cannot be directly combined, the large body of comparative data available in the literature often provides a helpful background for interpretation of isozyme data.

[4] C. J. Bult and Y.-T. Kiang, this volume [6].
[5] J. Britton-Davidian, this volume [7].
[6] R. W. Murphy, J. W. Sites, Jr., D. G. Buth, and C. H. Haufler, *in* "Molecular Systematics" (D. M. Hillis and C. Moritz, eds.), p. 45. Sinauer, Sunderland, Massachusetts, 1990.
[7] D. Goldman and S. J. O'Brien, this volume [8].

An important aspect of isozyme work is that provision must be made for the careful handling of tissues to keep them solidly frozen at all times. Proteins are particularly sensitive to thawing events. This means that dry ice or liquid nitrogen procedures must be used during the collection and transportation of the tissues; in addition, at least a $-70°$ freezer is needed for the storage of tissues.

Comparative Immunological Methods

A number of immunological techniques have been used in comparative studies.[8,9] The most important of these is microcomplement fixation (MC'F), a quantitative technique that has played a key role in many classic studies of molecular evolution and molecular systematics. By selecting proteins with different rates of evolution, a broad range of divergences can be examined. The cost of the technique is moderate, but biochemical expertise is required and the labor involved is substantial. Protein must be purified from some or all taxa for antibody production, and, for those taxa, a sizable tissue or serum sample is needed. Antibody production itself is usually done in rabbits, so an animal care facility must be available. Like isozyme electrophoresis, the large body of immunological distance data already available ensures the continued value of this technique for certain investigations.

DNA–DNA Hybridization

Solution hybridization studies of DNA have been used extensively in the analysis of genome complexity and organization, and intensively in phylogenetic studies of some groups.[10,11] The technique has the attractive feature that it ideally allows all (or some significant fraction) of two genomes to be compared at once. It is relatively simple to perform in the laboratory, and neither the cost nor the time and labor involved is prohibitive for small to medium sized numbers of samples. From a physicochemical standpoint, however, the technique is much more complicated than it appears at first, because what is really taking place in the hybridization vessel is many thousands or millions of separate hybridization reactions, each with its own kinetics and thermal stability. Careful attention to experimental design, laboratory technique, and data interpretation is essential. Also, pairwise comparisons between all samples are often desirable,

[8] M. H. V. Van Regenmortel, C. Joisson, and C. Wetter, this volume [10].
[9] L. R. Maxson and R. D. Maxson, *in* "Molecular Systematics" (D. M. Hillis and C. Moritz, eds.), p. 127. Sinauer, Sunderland, Massachusetts, 1990.
[10] M. S. Springer and R. J. Britten, this volume [17].
[11] S. D. Werman, M. S. Springer, and R. J. Britten, *in* "Molecular Systematics" (D. M. Hillis and C. Moritz, eds.), p. 204. Sinauer, Sunderland, Massachusetts, 1990.

so the cost and labor involved goes up rapidly with the number of samples. Relatively large quantities of DNA are needed, which may be hard to come by for some smaller organisms or those with low DNA content per unit mass. Data can be compared to other studies in a general way, but adding new taxa to a data set generally will require making pairwise comparisons with some or all previously studied taxa.

Restriction Enzyme Analysis

The comparative analysis of restriction site maps or restriction fragment patterns is a powerful tool for evolutionary studies at lower taxonomic levels. The site mapping approach can be extended to somewhat higher levels of divergence than fragment comparisons. The fragment comparison approach is much less time-consuming than site mapping, but it may lead to difficulty in determining homology for fragments in regions that have undergone structural rearrangements or show high degrees of variability. Large numbers of samples can be analyzed routinely, and the biochemical complexity of the technique is moderate. There are several methodological approaches to restriction analysis (end labeling, Southern blotting, PCR followed by digestion); all are fairly expensive to perform. Well-purified, high molecular weight DNA is a prerequisite for the end labeling and Southern blotting methods. The mitochondrial and chloroplast genomes have been popular targets for restriction analysis; for such projects the organellar genome must be extensively purified, or cloned probes must be available. In these cases, it is desirable to have tissue samples rich in the organelle. For example, vertebrate blood is a poor source of mitochondrial DNA; liver or heart samples work much better. Similarly, young leaf samples are the best source of chloroplast DNA.

Random Amplification of Polymorphic DNA

Random amplification of polymorphic DNA (RAPD) is an *in vitro* amplification technique (see section on polymerase chain reaction below) that eliminates the need for any prior characterization of the genome to be analyzed.[12–14] Based on the random amplification of DNA segments using single short primers of arbitrary sequence, RAPD readily detects genetic polymorphisms in most organisms. The method is simple and rapid to

[12] B. M. Bowditch, D. G. Albright, J. Williams, and M. J. Braun, this volume [21].
[13] J. G. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey, *Nucleic Acids Res.* **18,** 6531 (1990).
[14] H. Hadrys, M. Balick, and B. Schierwater, *Mol. Ecol.* **1,** 55 (1992).

perform, and large numbers of individuals can be analyzed at a reasonable expense. Only small amounts of DNA are required, and the same set of primers can be used on any organism. The method does demand careful replication of reaction conditions to achieve consistent amplifications, and good quality (i.e., high molecular weight) DNA is probably desirable. RAPD markers have already been used extensively in gene mapping research, and they are likely to see regular use in population and species level studies, especially if the organisms in question are small, poorly known, or otherwise difficult to study.[15] There is some concern at present about their use in DNA typing or fingerprinting studies owing to the occasional appearance of nonparental bands in offspring of known parentage.[16] In phylogenetics, the method is likely to be useful only among very closely related organisms because of the rapid evolution of band patterns. Another limitation is that the majority of RAPD markers show dominance, so that heterozygotes cannot be distinguished from homozygotes for the dominant allele.

Nucleic Acid Sequencing

Nucleic acid sequencing reveals the discrete order of nucleotides that encode all genetic information. In principle, sequence data can provide the greatest possible resolution for most studies, yet sequencing is the most expensive and labor-intensive of the common comparative techniques. Therefore, other procedures should be considered first to determine whether they could be employed effectively to answer questions of interest. A key issue here is whether the sequence of a single gene is adequate to answer the question or whether a broader survey of the genome is needed. Sequencing should be considered especially when conservative genes need to be compared among very divergent organisms, or when the level of resolution obtained by other techniques is inadequate. Preparations for sequencing prior to the late 1980s typically involved extensive molecular cloning procedures, making it impractical for many comparative studies. PCR has short-circuited the process of producing purified template for sequencing and opened a new era in molecular evolutionary biology by making it feasible to determine and compare the exact nucleotide sequence of homologous genes for many dozens, even hundreds, of individuals or species. An especially attractive feature of sequence data is that, because it is determined absolutely, the sequence can freely be compared to any other alignable sequence determined by the same or any other investigator.

[15] R. C. Wilkerson, T. Parsons, D. G. Albright, T. A. Klein, and M. J. Braun, *Insect Mol. Biol.* in press (1993).

[16] M. F. Reidy, W. J. Hamilton III, and C. F. Aquadro, *Nucleic Acids Res.* **20**, 918 (1992).

An advance in DNA sequencing is the development of automated sequencing instruments that replace the traditional radioactive labels with laser-activated fluorescent labels and that read sequences from gels directly into computer files. The development of automatic sequencers is a promising new strategy for DNA sequencing that increases the speed of data acquisition, but they are still too expensive for most individual laboratories to be able to justify their purchase.[17]

Polymerase Chain Reaction

The polymerase chain reaction technique allows for the direct *in vitro* amplification of DNA. It is not itself a comparative technique but forms the basis for others. PCR has revolutionized molecular evolutionary studies by making it possible to amplify similar segments of DNA from many organisms quickly and simply. It is possible to accomplish in 1 day by PCR what would have taken months or years to do by standard cloning techniques. PCR methods have greatly extended the range of feasibility of molecular investigations beyond previous limits on the age, quality, and quantity of material available for study.[18,19] The technique is relatively simple in concept and practice. It requires little time, labor, and cost compared to the results achieved. Yet the method is so extraordinarily versatile that it has affected experimental strategies throughout molecular genetics. This is especially true of evolutionary research, where comparison of homologous genes is all-important.

Beside greatly facilitating techniques such as DNA sequencing and molecular cloning, PCR technology has spawned a number of new comparative techniques that are providing invaluable information for evolutionary research. One of these (RAPD) has been mentioned in some detail above. Others include methods for the analysis of polymorphism at hypervariable genetic loci such as variable number tandem repeats (minisatellites)[20] and di- or trinucleotide repeats (microsatellites).[21] The highly informative, single-locus genetic markers provided by these methods are finding wide application in studies requiring genetic mapping, individual identification, parentage analysis, or other forms of DNA typing (fingerprinting).

Despite its versatility, there are some limitations to PCR that should

[17] D. M. Hillis, A. Larson, S. K. Davis, and E. A. Zimmer, *in* "Molecular Systematics" (D. M. Hillis and C. Moritz, eds.), p. 318. Sinauer, Sunderland, Massachusetts, 1990.
[18] E. M. Golenberg, D. E. Gianasi, M. T. Clegg, C. J. Smiley, M. Durbin, D. Henderson, and G. Zirawski, *Nature (London)* **344,** 656 (1990).
[19] S. Pääbo, J. A. Gifford, and A. C. Wilson, *Nucleic Acids Res.* **16,** 9775 (1988).
[20] A. J. Jeffreys, R. Neumann, and V. Wilson, *Cell (Cambridge, Mass.)* **60,** 473 (1990).
[21] J. W. Weber and P. E. May, *Am. J. Hum. Genet.* **44,** 388 (1989).

be noted. For a specific target segment to be amplified, prior knowledge of the sequence of its termini must be available so that specific primers can be synthesized. With current methods, the enzymatic synthesis of DNA required for PCR is relatively inefficient for long molecules. Thus, amplification of segments longer than a few kilobases may be difficult. The exquisite sensitivity of the technique makes it particularly vulnerable to contamination, especially by previously amplified PCR products.[22] For this reason, a number of precautions are necessary in order to prevent contamination, and it is advisable to physically isolate pre-PCR from post-PCR protocols if at all possible (see section on space concerns below).[3,22] Finally, PCR amplifies all viable target molecules in a sample more or less indiscriminately. Thus, if the population of target molecules is heterogeneous, the PCR product is likely to be heterogeneous as well. This feature of PCR is advantageous in some situations[23] but disadvantageous in others.[24]

Molecular Cloning

Standard molecular cloning techniques provide a means for the dissection of complex genomes and the *in vivo* amplification of specific sequences.[25] In many cases, little or no prior characterization of the sequence of interest is necessary. Like PCR, cloning is not in itself a comparative technique but provides the foundation on which several of the other techniques rest. For example, restriction analysis often involves the use of cloned probes. DNA sequencing following cloning is a commonly used method that consistently results in high-quality sequencing gels. Cloning of PCR amplification products dramatically enhances the efficiency of cloning and reduces the need for extensive screening efforts to find clones containing the desired insert.

Molecular cloning involves the use of bacteria, bacteriophage, and plasmids; thus, attention to sterile technique is imperative, and a working knowledge of bacterial and phage genetics is useful. Of the techniques discussed here, it is the most demanding in terms of molecular genetic expertise. Both the cost and the labor involved vary according to the method employed, but can be substantial. Now that many genes have been well characterized from a variety of organisms, some comparative molecu-

[22] S. Kwok, *in* "PCR Protocols" (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.), p. 142. Academic Press, New York, 1990.
[23] S. Pääbo and A. C. Wilson, *Nature (London)* **334,** 387 (1988).
[24] D. A. Lawlor, C. D. Dickel, W. W. Hauswirth, and P. Parham, *Nature (London)* **349,** 785 (1991).
[25] S. L. Martin and H. A. Wichman, this volume [22].

lar laboratories will not find it necessary to attempt *de novo* cloning. Still, the many cloning strategies available today provide such exquisite control and flexibility in manipulating DNA that they are sure to remain the backbone of mainstream molecular genetics and allow the development of new comparative molecular approaches.

Assessing Environment and Resources

Once research goals have been set and comparative techniques chosen, the next critical step in planning a laboratory is assessing the local environment and the resources available for research. As discussed above, the comparative molecular techniques now available differ greatly in complexity as well as in the type and cost of equipment required. Some can be performed with minimal laboratory facilities on a modest budget, whereas others are likely to be implemented successfully only at institutions where a substantial amount of molecular genetics expertise and activity already exists. In some instances, a survey of existing resources will cause the researcher to reconsider decisions on goals or techniques (Fig. 1). It is important to assess resources in several general categories.

Physical Plant

The physical space designated to house a new laboratory may vary in its state of preparation for laboratory work. Ideally, the space will have been originally designed as a laboratory, but in some cases it will be little more than empty rooms. The space itself and the physical plant it is housed in should be viewed with the following questions in mind. Is adequate bench space and cabinetry already in place, or will it have to be installed? Are sinks and plumbing sufficient to support the contemplated work? Is purified water available? Is a fume hood present or available nearby? Does the general ventilation of the entire space meet applicable standards for laboratories? Is the electrical power supply sufficient? Will the number and location of receptacles allow flexibility in equipment location? Are appropriate high-capacity circuits available for large equipment?

Instrumentation

All molecular genetic techniques require some amount of specialized scientific equipment. Because many items of equipment are expensive, it is a good idea to determine what instrumentation exists at the department or institution level, and to what extent it can be shared. Table II provides information on equipment required for the various comparative techniques and the ease with which each instrument can be shared. Items that typically are shared include large equipment such as high-speed and ultra-

TABLE II
MAJOR EQUIPMENT NEEDED FOR COMPARATIVE MOLECULAR GENETICS LABORATORY[a]

Item	Isozyme electrophoresis	MC'F	DNA–DNA hybridization	Restriction analysis	RAPD	Sequencing	PCR	Cloning
Large Equipment								
Autoclave	n	o	o	s	o	o	o	s
Ultracentrifuge	n	o	o	s	o	o	o	s
High-speed centrifuge	n	i/s	i/s	i/s	i/s	i/s	i/s	i/s
Ultracold freezer (−70° or below)	i/s	i/s	i/s	i/s	i/s	i/s	i/s	i/s
Crushed ice machines	s	s	s	s	s	s	s	s
UV/visible spectrophotometer	n	s	s	s	s	s	s	s
Polaroid MP-4 camera or equivalent	s	n	o	s	s	s	s	s
UV transilluminator	n	n	o	s	s	s	s	s
Darkroom with film developing facilities	o	n	n	s	n	s	s	s
Scintillation counter	n	n	s	s	n	s	n	s
Shaking incubator (ambient to 65°)	n	o	o	o	o	n	n	n
Laminar flow hood	n	n	n	n	o	n	o	o
Digitizer	o	n	n	o	o	o	n	n
Oligonucleotide synthesizer	n	n	n	o	o/s	o/s	o/s	n
General Equipment								
Freezer (−20°)	i	i	i	i	i	i	i	i
Refrigerator	i	i	i	i	i	i	i	i
Low-speed centrifuge	o	i/s	i/s	o	o	o	o	i/s
Microcentrifuge	i	i/s	i/s	i	i	i	i	i
Oven (ambient to 200°)	n	i/s	i/s	i/s	i/s	i/s	i/s	i/s
Stationary incubator (ambient to 68°)	s	n	n	s	s	s	s	i
Shaking water bath (ambient to 68°)	n	i	s	o	n	n	n	n
Water bath (ambient to 95°)	s	i	i	i	i	i	i	i
Top-loading balance	i	i	i	i	i	i	i	i
Analytical balance	i/s	i/s	i/s	i/s	i/s	i/s	i/s	i/s
pH meter	i	i	i	i	i	i	i	i
Stirring hot plate	i	i	i/s	i	i	i	i	i
Electrical homogenizers, blenders	i	i/s	i/s	s	s	s	s	s
Geiger counter	n	n	i/s	i/s	n	i	n	i/s
Pipettors (20, 200, 1000 μl)	i	i	i	i	i	i	i	i
Power supply (250 V, 150 mA)	i	o	n	i	i	i	i	i
Power supply (2500–3000 V, 300 mA)	n	n	n	n	n	i	n	n
Gel boxes (several varieties)	i	i	n	i	i	i	i	i
X-Ray film cassettes	n	n	n	i	n	i	n	i
Intensifying screens	n	n	n	i	n	o	n	i
Gel dryer	o	n	n	n	o	i	n	n
Vacuum centrifuge dryer	n	n	o	o	o	o	o	o
DNA thermal cycler	n	n	n	o	i	o	i	o
Liquid nitrogen tank	s	o	s	s	s	s	s	s
Microwave oven	o	n	n	i	i	n	i	i
Heating block	o	n	o	i	n	i	n	o
Vortex	i	i	i	i	i	i	i	i
Vacuum pump	n	i/s	o	o	n	i	n	n

[a] i, Needed for individual laboratory; o, optional; s, needed on a shared access basis; n, not used for that technique.

centrifuges, scintillation counters, autoclaves, automatic dishwashers, darkrooms and associated photographic equipment, and coldrooms. Depending on the size and needs of both the new and preexisting laboratories, it may be convenient to share other items, such as spectrophotometers, thermal cyclers, and ultracold freezers.

Core Facilities and Support Services

Most research institutions offer at least some centralized support services. Common examples include animal care facilities, greenhouses, glassware washing, chemical stockrooms, radiation safety support, and hazardous waste disposal. It is important to assess not only the existence, but the quality and reliability of such services. A central computing facility, for example, exists at most institutions, but not all maintain access to genetic databases or software for analysis of DNA sequences. Licensing fees for the more comprehensive software packages may cost several thousand dollars annually. The precise services offered by the institution will often determine the extent to which the individual investigator must act independently.

A trend at larger institutions is to establish a core facility to provide access to some of the more expensive molecular genetic technologies on a "pay as you go" basis. Such facilities generally perform oligonucleotide synthesis to provide primers for PCR and sequencing. They may also offer DNA sequencing services, which, for a small project or pilot survey, may represent a cost-effective alternative to setting up an independent laboratory.

Human Resources

The wide range of molecular techniques available and the rapid pace of new developments make it difficult for an individual to stay apprised of all aspects of the field that might impinge on the research at hand. For this reason, other researchers involved in molecular genetics are a key element of the local environment. This may include other members of the same research group, scientists who share the laboratory facility, or others doing various kinds of molecular genetics research at the same or nearby institutions. The collective molecular expertise in the local environment (and how well one can hope to make use of it) should be carefully assessed, and it may influence decisions on the choice of technique and the scope of research envisioned.

In addition to the local intellectual environment, communication among researchers via electronic bulletin boards is becoming a common route to seeking answers to laboratory technical problems. Electronic mail subscription lists include such groups as a Molecular Evolution Discussion Group and a Methods and Reagents Discussion Group. Information about these bulletin boards can be obtained through standard LISTSERV subscription lists from BIOSCI@NET.BIO.NET or via USENET.[26]

[26] K. Hoover and D. Kristofferson, *Plant Mol. Biol. Rep.* **10,** 228 (1992).

Selecting Equipment

The techniques that are to be implemented will determine what equipment is necessary. Some equipment may already be available in or near the laboratory, but it is likely that other items will have to be purchased. Once it is clear that a certain instrument must be purchased, there are still many factors that will affect selection of the exact make and model best suited to a particular laboratory. Some key issues to keep in mind are the following. Is the model in question adequate to support the contemplated technique(s)? Will other techniques require the same or similar instruments? Will the particular model support those techniques as well? Is a more versatile model available? Does its versatility justify any additional cost? Can the instrument (and its associated costs) be shared? How reliable is the model in question? Are support and maintenance available from the vendor or other sources? How expensive is maintenance? Will changing technology or new research goals make a different model significantly more desirable in the near future?

Organizing the Laboratory

The physical organization of the laboratory will, of course, depend on many factors peculiar to a particular facility and research program. Despite the many differences among laboratories, however, some major considerations are common to all. The location and utilization of equipment need to be well thought out in terms of space and facilities available, potential technologies to be employed, safety concerns, and efficiency. The following discussion assumes that a preexisting laboratory space will be occupied with little opportunity for altering the placement of benches, sinks, hoods, etc. If the space is to be newly built or renovated for work, then a number of design issues come into play; these are briefly treated in the next section.

Space Concerns

Within the physical limitations imposed by a preexisting laboratory design, much can be done to streamline operations through careful placement of equipment and designation of dedicated areas for certain procedures. For example, the space allocated to the laboratory operation may be arranged in one or several rooms. If separate rooms are available, it may be desirable to dedicate one or more rooms to purposes that, for reasons of safety, quality control, or convenience, are logically separated from other laboratory functions. Radioisotope work is often carried out in an auxiliary room for safety reasons. Large items of equipment may be well placed in a separate room, especially if they generate noise or heat or if they need to be

shared. A small room adjoining the main laboratory is a good location for a camera stand and light box to record electrophoresis results, because the room lights can be turned out without interrupting other workers. Tissue collections and associated activities, a major commitment of many molecular systematics laboratories, can be placed in a room with few laboratory accoutrements, as long as precautions are taken to guard against freezer failure.

A growing number of comparative laboratories isolate pre-PCR and post-PCR procedures in separate rooms. Contamination of samples or reagents with even minute amounts of a previously amplified PCR product can be a serious problem, especially for those working with ancient or degraded DNA. Such products are guaranteed to contain perfect primer binding sites, and they may amplify better than the target DNA in a new sample. "Pre-PCR" protocols include sample storage, genomic DNA isolation, and the initial preparation of PCR reactions. "Post-PCR" protocols include PCR itself, purification and electrophoretic analysis of PCR products, DNA sequencing, and secondary amplifications.[3]

Another aspect of spatial organization will be decisions on allocation of personal bench space versus a "workstation" arrangement of laboratory bench tops. A standard space configuration of a single-investigator laboratory involves the designation of small personal bench spaces and the establishment of common bench areas for shared equipment and setup of space-consuming operations. However, comparative laboratory work involves moderate to high degrees of repetition, and sharing of molecular facilities among many investigators is becoming more common. Under these conditions, a workstation arrangement may be desirable, in which standardized protocols are performed at workstations which are outfitted and maintained for that express purpose.

Facilities Considerations

The exact configuration of utilities and services within the laboratory will play a role in determining optimal organization. For example, the position of a fume hood may be the decisive factor in choosing where DNA extractions and other protocols involving organic solvents are carried out. This in turn may dictate placement of water baths and centrifuges used in DNA extraction. The position and size of sinks should be considered as well. Inherently messy protocols, such as Southern blotting and DNA sequencing, should take place near sinks to minimize problems with spills and cleanup. Electrical receptacles and the capacity of circuits will also play a role in the positioning of equipment. Equipment requiring voltages other than the standard line voltage will need special circuits and recepta-

cles. Many pieces of laboratory equipment have significant amperage demands, so care must be taken not to overload circuits. This may mean that apparatuses with heating or cooling elements need to be spaced evenly around the laboratory.

Beyond simple issues of equipment placement, power and plumbing can be serious problems within the laboratory infrastructure. With the increased use of computer technology and the development of sophisticated equipment, the quality of electrical power needed for laboratory use has risen. Power surges and sags will shut down computers and computer-operated equipment, and low voltages will stress and wear relay switches and solenoids in machinery. Surge protectors can be useful for most electric spikes and sags. Some equipment, such as water baths and incubators, restart after a short power loss. Other apparatuses, such as ultracentrifuges and ultrafreezers, will restart but are vulnerable to damage from voltage fluctuations. Electronic alarms, backup cooling systems, and a predetermined strategy for responding to freezer failure are all highly desirable. It is notable that most PCR machines do not restart automatically, and interruption of the run may spell a total loss of the reactions and time involved. Long computer analysis programs also will be irreversibly interrupted. Uninterruptable power sources can be purchased that will clean the power and switch to a battery backup within nanoseconds if necessary.

Plumbing can become a significant concern in molecular laboratories, and thought must be given to designating sinks for specific purposes. A large sink 3 to 5 feet long and 12 to 18 inches deep will be wanted for glassware washing and DNA sequencing purposes. On one sink, it may be useful to have a second faucet installed so that one of the faucets may be permanently connected to an aspirator pump for pulling vacuums. In addition, purified water (distilled and/or deionized and charcoal filtered) is required for accurate quantification of sensitive reactions. House-distilled water in some older installations may not be of the quality required. A purification system can be installed at individual sinks to supplement house distillation if needed.

Occasionally, a researcher will be asked to participate in the design of new laboratory facilities or substantial renovation to existing facilities. Although a detailed discussion of laboratory design is beyond the scope of this chapter, a few crucial issues can be stressed. First, seek architectural, engineering, and construction firms with experience in laboratory design and construction. This is a highly specialized area that presents technical problems new to many firms. Second, plan on investing much time and effort in the process. Work closely with the architects, engineers, and builders to familiarize yourself with the design process from the ground up.

Make sure they understand the needs and expectations of the end users of the facility. Third, do not assume anything will be automatically "taken care of." Ask many questions. Things that seem obvious to you may not be obvious to the architect, and minor oversights, especially in the early stages, can result in major problems later. Fourth, remember that you will have to live with the result. The architects, engineers, and institutional administrators will move on to other projects, but you and your colleagues will have to work in the resulting facility, good or bad, for years to come.

Organizing around Techniques

With multiple operations often occurring simultaneously, the organization of laboratory equipment needs to accommodate both the grouping of equipment by technique and the sharing of equipment among the different technologies. It is often the case in molecular evolutionary laboratories that several researchers will carry out similar research programs using the same molecules (or genes) and similar methods on different organisms. This requires multiple users to share similar sets of experimental facilities. Laboratory areas are most efficiently organized with designated areas for major techniques such as nucleic acid isolations, Southern blotting, PCR, and sequencing. This situation lends itself to the workstation concept mentioned above. A DNA sequencing workstation, for example, would typically include bench space for pouring gels, one or two gel rigs and associated power supplies, a gel drying unit and associated vacuum pump (aspirator type), and a variety of sequencing paraphernalia, all located near a large sink for washing the large glass plates used for pouring gels. Careful organization of stations around the techniques to be performed there can result in significant streamlining of research.

Safety

Safety concerns revolve around general laboratory safety, hazardous waste management, and radiation safety. Included in any laboratory operation should be the recognition of the potential hazards associated with the laboratory work space, and training in safety procedures should be included in the orientation of new laboratory members. Work with organic solvents or other noxious materials that are potentially volatile or aerosol-forming should be carried out in a fume hood. Emergency showers and eyewashes should be available in each section of the laboratory. Working with aqueous solutions and electrical equipment presents the constant hazard of electrical shock. To minimize this hazard, all electrophoresis apparatuses should be fitted with safety shields that cover buffer compart-

ments during use, and circuits servicing areas near sinks should have ground fault circuit interrupters installed.

Use and disposal of potentially hazardous chemicals are becoming heavily regulated. Proper storage, maintenance of Material Safety Data Sheets (MSDS) about each chemical, and the control and documentation of hazardous waste disposal are among the required responses to laboratory safety. An institution Safety Officer can provide necessary information about reporting and disposal requirements.

Reagents labeled with radioactive isotopes (^{32}P, ^{35}S, ^{3}H) are commonly used in molecular biology despite recent development of chemiluminescent labeling techniques.[26,27] Obtaining appropriate licensing, working behind shields, monitoring work areas with radiation detectors, and disposing of radioactive waste properly are aspects of this work. Institutions with researchers using radioactive materials often employ a Radiation Safety Officer who will be cognizant of the local and federal rules and regulations. Clearly, close cooperation with this person is important and will result in the most satisfactory working conditions.

Planning for the Future

In attempting to equip and organize a comparative molecular laboratory, one must keep in mind that molecular biology is an extremely dynamic field. The rapid pace of technological innovation makes change the rule rather than the exception. A well-planned laboratory facility will have the capacity for change built into it. Standard features of modern design which contribute to flexibility include gridded drop ceilings that provide access to utilities and services and modular bench units that can be easily rearranged as the need arises. Even quite simple measures, such as buying tables with lockable wheels and installing extra circuits and receptacles so that electrical power is readily available throughout, can make the laboratory space substantially more adaptable to the demands of new technology.

Change affects not only methodological aspects of laboratory work but equipment requirements as well. For example, thermal cyclers are a necessity in molecular laboratories today but were unheard of before the advent of PCR. PCR technology has also made it possible to do a good deal of comparative molecular work without the aid of ultracentrifuges or steam sterilizers, items that are expensive but irreplaceable components of a standard molecular genetics laboratory. Clearly, it behooves the researcher

[27] P. M. Gillevet, *Nature (London)* **348,** 657 (1990).

to stay as current as possible with technological developments in order to anticipate changing demands on space and equipment.

Several recent innovations seem likely to have an impact on comparative molecular laboratories. Nonradioactive DNA labeling techniques promise to reduce the need for radioisotopes and associated safety concerns.[27,28] New applications of PCR continue to appear at a dizzying rate. These methods are sure to increase the need for thermal cyclers. Perhaps the most dramatic changes will come in laboratory automation. Automated instruments for the isolation of nucleic acids from various biological samples are available from several sources. Automated laboratory workstations equipped with industrial robot systems are also available commercially. Digital scanning systems allow automated capture and interpretation of data from two-dimensional images such as autoradiographs of restriction fragment length polymorphism (RFLP) or DNA sequencing gels. Automated DNA sequencers that combine gel electrophoresis with data capture have been available for several years. It seems likely that complete automation of DNA sequencing, from template preparation to direct data acquisition into computer files, will be possible. The potential effect that such extensive automation could have on molecular evolutionary research would be dramatic. Although laboratory automation is in its infancy, it is a rapidly growing field to which close attention should be given.

Finally, it should be noted that it is possible to overreact to new technology. The constant onslaught of new techniques and apparatus can be fascinating, and the temptation to switch to the latest (and trendiest) thing can be strong. However, the ultimate goal of answering evolutionary questions must be kept in mind, and the value of older techniques should not be underestimated. The real issue is how well a particular approach addresses the problem at hand. Sometimes an older, more mundane technique actually provides a better solution than the latest innovation. Many of the currently available techniques are likely to provide reliable, cost-effective approaches to questions of evolutionary interest for years to come.

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[28] G. M. Church and S. Kietter-Higgins, *Science* **240,** 185 (1988).