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VIEWPOINT

Applied Biotechnology in Nematology

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Abstract: During the past two decades, rapid advances in biotechnology and molecular biology have affected the understanding and treatment of human and plant diseases. The human and *Caenorhabditis elegans* genome-sequencing projects promise further techniques and results useful to applied nematology. Of course, biotechnology is not a panacea for nematological problems, but it provides many powerful tools that have potential use in applied biology and nematode management. The tools will facilitate research on a range of previously intractable problems in nematology, from identification of species and pathotypes to the development of resistant cultivars that have been inaccessible because of technical limitations. However, to those unfamiliar or not directly involved with the new technologies and their extensive terminology, the benefits of the advances in biotechnology may not be readily discerned. The sustainable agriculture of the future will require ecology-based management, and successful integrated nematode management will depend on combinations of control tactics to reduce nematode numbers. In this review we discuss how biotechnology may influence nematode management, define terminology relative to potential applications, and present current and future avenues of research in applied nematology, including species identification, race and pathotype identification, development of resistant cultivars, definition of nematode–host interactions, nematode population dynamics, establishment of optimal rotations, the ecology of biological control and development of useful biological control agents, and the design of novel nematicides.

Key words: agriculture, applied biotechnology, biological control, biotechnology, identification, management, molecular biology, nematode.

An extension nematologist receives a sample containing an unknown root-knot nematode. Based on **isozyme** (refer to glossary for definitions of boldface terms) analyses of female nematodes and **mitochondrial DNA (mtDNA)** polymorphism in second-stage juveniles (J2), the species identities of 20 female and 100 J2 are ascertained within hours. The extension nematologist finds that the field in question contains a mixture of 60% *Meloidogyne incognita* and 40% *M. arenaria.* This scenario, which would have been implausible 10 years ago, can occur today because of advances in biotechnology. It is likely that within the next few years, the extension nematologist in our story will also establish the nematode race designation and determine whether the *M. incognita* is a pathotype capable of parasitizing root-knot resistant tomato cultivars with the *Mi* gene.

During the past two decades, rapid advances in molecular biology have affected understanding and treatment of human and plant diseases. The human and *Caenorhabditis elegans* genome **sequencing** projects (41,52) will yield additional useful techniques and results. Important as the new molecular techniques and tools are to basic biological research, they have use in applied biology as well, and nematode management will definitely benefit. However, to those not directly involved in and unfamiliar with the new technologies and their terminology, the benefits of the ad-

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vances are not obvious because of technical jargon. A short glossary is included at the end of this paper to assist with terminology, and the first appearance of each glossary term in the text is emphasized with bold-face type. Despite the sometimes confusing language, most of the principles and ideas of molecular biology are simple. The details are often complex, but the concepts are easily understood and are becoming more accessible to the users of the technology.

Biotechnology is not a panacea for nematological problems, but it provides many powerful tools. These tools will be helpful in many areas of nematology, including species identification, race and pathotype identification, development of resistant cultivars, definition of nematode–host interactions, nematode population dynamics, establishment of optimal rotations, the ecology of biological control and development of useful biological control agents, and the design of new nematicides (13). By presenting examples, we attempt to address the questions of what the priority areas are for biotechnology and molecular biology in applied nematology, and how new techniques will illuminate old questions and eventually benefit the practicing nematologist or extension agent.

All the potential applications of biotechnology to nematology cannot be covered here, nor will there be an attempt to present detailed summaries of new techniques. Rather, our goals are to highlight the areas this technology can and will influence in applied nematology, to present current and future avenues of research in applied nematology, and to convey our excitement for the prospects of future advances in applied nematology. The references we have selected provide an entrance to the burgeoning literature, but are intended as only an introduction. Many of the subjects discussed herein have not yet been attempted in nematology, but we expect that they will be. The necessary tools are available, and most of what we present can be done. Much of it probably will be.

Nematode Species Identification

Accurate identification of nematode species is the foundation of nematological research, quarantine enforcement for regulatory purposes, and nematode management, especially management that does not rely on nematicides. An immediate objective of new techniques in nematology is to increase the ease, accuracy, and speed of species identification (58). The use of molecular markers to identify nematode species increases the detection sensitivity in samples, which is important for regulatory nematology, and will eventually increase the speed of quantifying nematode species found in research or management samples.

Isozyme electrophoresis and antibodies: Root-knot (*Meloidogyne* spp.) and cyst nematodes (*Globodera* spp., *Heterodera* spp.) are sometimes difficult to identify using morphological characters, so alternative identification methods that are easily interpreted are desirable. It is now possible to distinguish among single adult females of *Meloidogyne arenaria, M. javanica, M. incognita, M. hapla, M. chitwoodi, M. naasi, M. exigua,* and *M. graminicola* based on isozyme differences in esterase and malate dehydrogenase (17,18). Many diagnostic laboratories now routinely use these isozymes to aid in root-knot nematode identification. Variation in esterase patterns have allowed identification of different forms or isolates of *M. incognita* and *M. arenaria* (12), although the relationship of the forms to race designations is unclear. However, there are limits to isozyme analysis: enzyme expression may vary relative to environment and nematode life stage, single J2 do not contain enough enzyme to allow their identification; and isozyme variants have not allowed successful identification of intraspecific variation among populations or isolates. Attempts to use antibodies to differentiate nematode species have been successful in some instances. *Globodera rostochiensis* and *G. pallida* can be identified with monoclonal antibodies (50).

DNA markers: The blueprint for an or-

ganism resides in its genome, its DNA. Accordingly, DNA ultimately determines a nematode's identity, and the DNA does not change with environmental conditions during a nematode's lifetime. Consequently, DNA markers are stable, and thus useful when applied to identifying nematode species and populations. Various DNA polymorphisms that allow identification of the major root-knot nematode species have been described (26,45,46).

Cyst nematodes are typically identified by host range and morphology; thus, identification of closely related cyst nematode species and pathotypes is difficult and time-consuming. Molecular techniques have provided new approaches to these problems, and the technique most often used for discriminating species of cyst nematodes is DNA analysis. *Globodera rostochiensis* and *G. pallida* can be identified by appropriate DNA **probes** (8).

Polymerase chain reaction (PCR): PCR is a recently developed technique that has been of particular consequence to molecular genetics because it allows amplification of specific sequences from minute quantities of DNA. In PCR two **oligonucleotide primers** are used to **hybridize** to opposite DNA strands flanking the desired sequence in the target DNA, so that **DNA polymerase** can create a copy of the intervening target DNA (37,38,49,61). The exponential amplification mediated by cycles of heating and cooling can produce 10^6–10^9 copies of the target DNA sequence (37,61). The technique is effective with samples containing little biomass; for example, it has been used to amplify DNA from single human sperm (14) or from blood stains (56).

Restriction fragment length polymorphisms (**RFLPs**) generated after PCR amplification of mitochondrial DNA (mtDNA) can be used for identification of single root-knot nematode J2 (46). The technique uses PCR and primers for the mtDNA of *Meloidogyne* spp. to generate DNA fragments that are subsequently subjected to restriction endonuclease digestion, yielding species-specific RFLPs that allow discrimination of *M. arenaria, M. chitwoodi, M. incognita, M. javanica,* and *M. hapla* (46). Using a similar approach, Vrain et al. (55) used PCR to amplify a portion of **rDNA** that contains RFLPs, which allowed differentiation of members of the *Xiphinema americanum* group.

Random amplification of polymorphic DNA (RAPD): Assaying the entire genome for DNA markers is a powerful way to obtain markers linked to characters of interest. The RAPD assay refers to PCR amplification of target DNA with single primers of arbitrary nucleotide sequence, and hence produces DNA fragments distributed over the entire target DNA pool (57). Because RAPD analysis uses random primers for PCR, foreknowledge of the nucleotide sequence information comprising the nematode genome is not required; because RAPD analysis uses PCR technology, it requires only tiny amounts of DNA and can be applied to single juveniles or single cysts (10,11).

The identification of root-knot nematode J2 is especially valuable, because J2 are the stage extracted from soil samples but are difficult or impossible to identify to the species level with traditional techniques. RAPD analysis has been used to successfully distinguish *M. arenaria, M. incognita, M. javanica,* and *M. hapla* (11); however, because this technique is sensitive to operator effects that cause variation in banding patterns, identification across different laboratories is difficult. Thus, it is desirable to **clone** species-specific RAPD markers, sequence the clones, and then synthesize larger, more stable primers that allow reliable amplification of the diagnostic bands (44).

Detection of infraspecific variation: Genetic variability in nematode populations, their hosts, and their antagonists is the stuff of which evolution is made. The variation that exists within nematode populations is just beginning to be documented (9), because genetic variability among nematodes was not especially important while nematicides were readily available and, more importantly, because the tools for assessing

genotypic variation have become available only during the past 10 to 20 years. For each nematode species, genetic markers associated with host range or life history characters (such as reproductive rates and survival characteristics) would be valuable in developing management practices that reduce nematode pathogenicity and **fitness** by allowing monitoring and selection for specific genotypes.

Geographic isolates of some nematode species, such as root-knot (*Meloidogyne* spp.), cyst (*Heterodera* spp. and *Globodera* spp.), and the stem-and-bulb nematode (*Ditylenchus* spp.) have unique characteristics. Isolates that are identified on the basis of their unique host ranges are usually termed "races," whereas isolates capable of circumventing known resistance genes are termed "pathotypes," although debate continues concerning a uniform terminology for infraspecific variants.

Variability has been widely recognized in *Meloidogyne* spp., with morphometric, reproductive, physiological, and cytogenetic variants observed on different host plants (39,42,48). Variation in pathogenicity and reproduction on 10 different hosts was observed among 12 populations of *M. arenaria* race 1 (42). The next challenge in the application of the new technologies is to obtain DNA polymorphisms useful as markers to identify pathotypes, races, and geographic isolates of *Meloidogyne* spp., and several researchers are looking for such markers.

DNA probes obtained from repetitive sequences identified in *Globodera rostochiensis, G. pallida,* and the *G. pallida* Pa1 pathotype are diagnostic and are used for the direct identification of individual cysts of these species by **dot blot** procedures (51). Markers obtained by RAPD analysis have allowed discrimination of geographic isolates of *Heterodera schachtii* (10), and RAPD analyses may aid in identifying DNA markers to distinguish cyst and root-knot nematode races and pathotypes. Other nematode species and host races that are not easily identified include the sibling species of burrowing nematodes (*Radopholus similis* and *R. citrophilus*). Efforts are under way to use RFLPs, rDNA, and RAPD to provide markers that differentiate burrowing nematode species and races (D. T. Kaplan, pers. comm.).

Nematode taxonomy and phylogenetics: Nematode morphology has been the basis of nematode taxonomy and hypotheses of nematode phylogeny; however, the homology of morphological characters that appear to be similar may be uncertain (27,36). Molecular techniques, especially DNA sequence comparisons, are being used to examine relationships among taxa, even among diverse taxa that cannot readily be compared with morphological analysis. Direct comparison of genotypes eliminates environmentally induced phenotypic variation; consequently, RFLP and DNA sequence analyses have proliferated in systematics, with comparisons of mtDNA and rDNA sequences playing a particularly important role (16,23,27,36). Several genes have conserved biochemical functions, such as those encoded by rDNA. Such genes occur in all species, so they can be sequenced, aligned, and analyzed to construct robust phylogenetic hypotheses (16,23). It appears that rDNA will be useful in assessing evolutionary relationships among related species and genera of cyst nematodes, although not necessarily for infraspecific relationships or closely related species (19). Sequence data from rDNA suggest that *Heterodera glycines, H. schachtii,* and *H. trifolii* are very similar, but that the three species are very different from *Heterodera avenae* (19). Comparison of the mtDNA nucleotide sequences of *Caenorhabditis elegans* and *Ascaris suum* suggests that the ancestral lines of these two species diverged about 80 million years ago, much more recently than some had postulated (43). The comparisons among *Heterodera* spp. and of *C. elegans* with *A. suum* are difficult based solely on morphological data. Including DNA data in phylogenetic analyses improves researchers' capacity to assess relationships among distantly related taxa.

Nematode Population Dynamics and Management

Competition and selection: Frequently, several root-knot nematode species occur in the same field. For example, *M. arenaria, M. incognita,* or *M. javanica* often occur together in fields in the southeastern United States and render development of cropping systems to suppress root-knot nematodes difficult. Part of the difficulty lies in defining the species that are present and their relative proportions, before and after rotations. Certain hosts in a rotation series will favor increase of one of the root-knot nematodes, and it is difficult to sample such fields accurately with morphology-based nematode identification. The use of isozymes to identify female root-knot nematode species is helpful, although the necessity for first obtaining females from the roots involves considerable work. A more convenient and more objective approach is the use of RAPD or mtDNA markers that allow identification of single J2 obtained from soil samples (11,46). Markers that can be assayed in single juveniles allow determination of relative numbers of species in a sample, so the selection pressures imposed on mixed nematode populations by different cropping sequences can be evaluated. Using standard techniques, such an assessment would be nearly impossible.

If the population in a field is a mixture of races or pathotypes, the situation is more difficult. The classical identification of races or pathotypes typically involves a bioassay including several plant species. In this situation, because race status cannot be assigned to an individual nematode (40), the frequency of races within a population cannot be determined. Rather, the population in the field is characterized as a certain pathotype or race on the basis of the average phenotype observed in a host-range test. Identification of DNA markers closely linked to nematode virulence or parasitism genes will allow investigators to assign pathotype, race, or biotype status to individual nematodes, facilitating determination of allele frequency in the population.

Nematode biogeography: Determination of the geographic center of origin of nematode species is valuable in the search for coevolved antagonists or sources of plant resistance. The geographic origin of nematode species is important in phylogenetics and regulatory nematology. In the past, it has been very difficult to determine the geographic origins of nematode species, and most speculations on origins have been based on host-plant biogeography. Adoption of DNA markers and mtDNA or rDNA sequence data to assess nematode biogeography is changing this situation, and, although in its infancy, the molecular approach will undoubtedly yield major insights.

Nematode–Host Interactions

The successful initiation and establishment of a feeding site by root-knot, cyst, and other sedentary endoparasitic nematodes involve a complex series of molecular signals and responses between nematode and host that lead to the development of specialized giant cells or syncytia in the host root. The exact means by which a nematode induces a susceptible response in its host is unknown, as are the gene(s) involved in plant resistance to nematodes.

Nematode resistance has been identified in different hosts, but there is little information available on virulence or parasitism genes in nematodes. The virulence genes in *Globodera rostochiensis* that correspond to the H1 resistance gene in *Solanum tuberosum* are being investigated with RFLP analysis of virulent nematode lines (2). The RFLP analyses will be used to construct a linkage map to isolate the nematode gene(s).

Nematode esophageal-gland secretions are thought to be involved in the induction of syncytia, and although the identification and characterization of the secretions is incomplete, they appear to include conjugated proteins (2,25). It is possible that small variations in the genes encoding the secretions determine the phenotypic dif-

ferences among races or pathotypes (25). Researchers are developing monoclonal antibodies specific to secretory proteins from *Meloidogyne* (25) and *Globodera* (2). An objective of one research group is to clone genes for monoclonal antibodies specific to *Globodera* esophageal-gland secretions and then insert the antibody genes into potato to obtain resistance (2). The concept is that expression of the monoclonal antibodies in plant cells will block the syncytium-inducing function of the secretions (2). Antibodies may help elucidate the function of secretory proteins, determine the cellular location of interactions between nematode secretory proteins and the host, and help to identify nematode genes for secretory proteins. Further elucidation of the nature of nematode esophageal gland secretions will assist in discovering the molecular basis of the host–parasite interaction.

Inhibiting the host–parasite interaction: Changes in gene expression in nematode-infected roots have been documented in several laboratories (7,20,60). Once the molecular interaction that initiates food cells is elucidated, there are many ways to use the information to interfere with the communication between nematode and host. Information on the genetic basis of the host–nematode interaction will allow the design of drugs or chemicals that interfere with transcription factors and block host gene expression necessary for nematode support. Disrupting translation with antisense **mRNA** that will bind to and inactivate target mRNA or designing catalytic RNAs (ribozymes) that destroy nematode-induced mRNAs are other ways to inhibit the host–nematode relationship (7,19). Molecules that interfere with membrane-bound receptors involved in initial chemical interaction between host and nematode could be designed (22). Finally, it may be possible to design or discover molecules that interfere with the signal transduction pathways necessary for the continued maintenance of giant cells or syncytia (5,19).

Host Resistance to Plant-Parasitic Nematodes

Many plant cultivars are resistant to certain plant-parasitic nematodes, but most of the available resistance is to cyst and root-knot nematodes. There is much plant germplasm that has not been tested for resistance to nematodes, especially the migratory endo- and ectoparasitic forms. In addition, resistance may function against various nematode life stages (21), a fact not considered in most resistance bioassays. Because very little plant germplasm has been subjected to adequate resistance bioassays, the germplasm available for further resistance screening is greater than might be immediately apparent.

Identification of resistance genes: Instead of conducting lengthy bioassays, breeders can assess the transfer of resistance genes in a cross by monitoring transmission of markers tightly linked to the resistance (34). The linked markers speed the breeding process and allow resistant cultivars to enter the marketplace sooner. For example, the introgression of root-knot nematode resistance into useful tomato cultivars was facilitated by the **linkage** between the resistance gene *Mi,* which confers resistance to *M. arenaria, M. incognita,* and *M. javanica* (59,60), and an isozyme of acid phosphatase (47). The close linkage allowed breeders to monitor the presence of this marker to verify *Mi* transfer to progeny seedlings and reduced the need to challenge plants with nematodes to assay for resistance (60). DNA markers discovered in tomato, which are more closely linked to the *Mi* gene than are isozyme markers, and a marker linked to *Heterodera schachtii* resistance in sugar beet, are replacing isozymes as preferred markers in breeding programs (30,60).

The tools of molecular biology are also being used to identify and clone nematode resistance genes, and the closest to being cloned is the *Mi* gene from tomato. The availability of **yeast artificial chromosome (YAC) libraries** (6) of the tomato genome

(35) and DNA markers closely linked to *Mi* (24) assist **cloning** efforts. A benefit of cloning resistance genes is the possibility of transferring resistance to crops without available resistance (22,59). In addition, pyramiding several different major resistance genes within one plant by classical breeding strategies is laborious and difficult, partially because of the agronomically undesirable characteristics often linked to resistance genes. Combining major resistance genes in one cultivar becomes a possibility with molecular methods. Such combinations of resistance could provide efficacy against a wide range of nematode species or pathotypes, giving more durable resistance. The search for the *Mi* gene is important on other levels as well. Once the *Mi* gene is identified, identification and characterization of its product(s) will improve knowledge of the molecular basis of host–parasite interactions and allow comparison or modification of resistance mechanisms.

Engineering new types of resistance: Molecular biology presents intriguing options for engineering new types of resistance. One approach is to take known resistance genes and subject them to recurrent bouts of mutation and selection to find new, improved variants (29). This "directed molecular evolution" approach has been used to change the normal function of RNA enzymes **(ribozymes)** so that they cleave DNA instead of their normal substrate, RNA (3). Directed molecular evolution may have potential for creating and identifying new nematode resistance genes but is contingent on first identifying resistance genes or gene products, a feat that is not too distant, perhaps 2 to 4 years (59).

Another possibility for enhancing plant resistance to nematodes is the construction of new resistance genes. Genes with regulatory elements that direct expression only in giant cells is a possibility. Such genes, under the appropriate conditions of giant cell or syncytium induction, can express toxins, growth regulators, antibodies, enzymes, or hormones inhibitory or toxic to nematodes, or gene products that alter the plant metabolic pathways necessary to sustain nematodes (7). The molecular basis of signal transduction in compatible nematode–host interactions is an important area of investigation, in which advances are being made (25).

Biological Control

Identification of antagonists and of active antagonist isolates: As with their nematode hosts, biochemical or molecular markers can aid in identifying antagonist species, potentially without requiring the isolation and culture of the antagonists (1). Within antagonist species that have potential for biological control, there are often differences among geographic isolates. Host specificity varies among individual populations or isolates of *Pasteuria penetrans,* and such specificity may result from differences in the amount and type of spore surface proteins (15). The exact mechanisms responsible for host specificity in *P. penetrans* are not clearly established, but DNA markers correlated with host specificity may aid in defining the mechanisms.

Antagonist isolates can differ in characteristics other than host range. Isolates of *Verticillium chlamydosporium* from *Heterodera avenae* eggs differ in pathogenicity to *H. avenae* eggs, chlamydospore production, growth rate, and optimum temperature for growth (31). Accordingly, *V. chlamydosporium* isolates, or isolates of any nematode antagonist, destined for use in biological control programs must be carefully selected on the basis of desirable phenotypes (31). Biochemical or molecular markers could be used to identify biologically active isolates that have desirable characteristics, such as pathogenicity or survival capacity. Although such markers are not yet available, the potential exists. For example, electrophoretic analysis of isozymes supported the separation of *Hirsutella thompsonii,* a pathogen of mites, into groups that agree with the morphological scheme that is the basis for separating *H. thompsonii* into three varieties (4).

Survival and movement of introduced antagonists: The identification of molecular markers specific to species or isolates of nematode antagonists will allow monitoring of the establishment, survival, growth, and spread of antagonists introduced into fields. Markers that are sufficiently specific to permit detection of antagonists without requiring isolation and culturing will be of particular value.

The epidemiology of soil-borne antagonists is poorly understood, but the development of markers will also assist in this regard. The utility of DNA markers and DNA–DNA hybridization to detect bacteria introduced into soils has been demonstrated (28,53,54) and should be applicable in nematode biological control to follow the spread and establishment of introduced antagonists.

Ecology of rhizosphere antagonists: The involvement of rhizosphere-colonizing microorganisms in mediating the antagonistic influence of certain cover crops towards plant-parasitic nematodes has been recognized (32,33). Some cover crops antagonistic to *Meloidogyne incognita* and *Heterodera glycines* host a distinct rhizosphere microflora compared with soybean, which is a host for both nematodes. Approximately four to six times the number of bacterial isolates from the cover crops, compared with bacteria isolated from soybean rhizospheres, reduced disease symptoms of *M. incognita* and *H. glycines*. Determination of the molecular basis of root colonization and nematode antagonism in rhizosphere bacteria may allow further development of the microorganisms as biological control agents, or allow transfer of genes conferring antagonism or rhizosphere competence to other nematode antagonists. As more is learned about the mechanisms of host specificity and virulence among nematode antagonists, the possibility of engineering strains with improved antagonistic potential increases.

Novel Nematicides

The design of environmentally acceptable novel nematicides specifically toxic to nematodes would reduce disruption of other soil fauna and native biological control organisms. Such specific toxins might mimic or inhibit expression of physiologically active nematode metabolites, hormones, or pheromones. The design of nematicidal compounds that are easily metabolized by soil bacteria to decrease nematicide half-life in the environment, or of microbiological delivery systems for nematicides using engineered rhizosphere-colonizing bacteria, are not farfetched goals. Synthesis of nematicidal or nematistatic compounds by rhizosphere-colonizing bacteria would assure that the toxins or pheromones that incapacitate or confuse nematodes are expressed where they are needed, near the roots.

Conclusion

There are many exciting research opportunities in applied and basic nematology that utilize the new approaches and tools of molecular biology. The tools will facilitate research on previously intractable questions in nematode biology that have been inaccessible because of technical limitations. Current nematode control options include the use of nematicides, cultural practices, resistant cultivars, and crop rotations. In the future, successful integrated nematode management will depend on combinations of control tactics to reduce nematode numbers. Application of biotechnology to nematode control tactics will influence applied nematology in diverse ways, from nematode identification to the development of resistant cultivars, and will improve effectiveness and increase the number of management options available. Increased communication and cooperation between researchers with expertise in the new technologies and the researchers with expertise in applied science is necessary to enlarge our understanding of nematode biology and ecology. The outcome will enhance development of new nematode management strategies and allow refinement of old techniques.

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Glossary

Definitions derived or adapted from King, R. C., and W. D. Stansfield. 1990. A dictionary of genetics, 4th ed. Oxford: Oxford University Press.

Clone—Single DNA segments from one organism, maintained in a larger **"vector"** DNA that is replicated within a microorganism, usually *Escherichia coli.*

Cloning—the formation of DNA clones or genetically identical replicas.

Dot blot—a technique in which spots or dots of target DNA (or RNA) denatured to single strands are bound to a membrane and then exposed to another single-stranded DNA (or RNA) that is labeled (a probe). If the target and probe share common sequences, they will bind to each other to form labeled, double-stranded molecules bound on the membrane where the target DNA was spotted. Spots homologous to the DNA probe are visualized by autoradiography or colorometric techniques.

DNA polymerase—an enzyme that catalyses the creation of a new DNA strand from an existing single-stranded DNA template using deoxyribonucleoside triphosphates.

Fitness—a property of a class of individuals that share certain genetic characteristics. It is a relative measure of an organism's ability to survive and contribute genes to the next generation under given environmental conditions, and is typically measured over one generation.

Hybridize—to pair single DNA strands that are identical in sequence, or to pair "complementary" single RNA and DNA strands to produce an RNA–DNA hybrid.

Isozymes—multiple forms of a single enzyme. Isozymes catalyze the same chemical reactions but can be separated by electrophoresis due to differences in amino acids that make up the polypeptide subunits.

kb (kilobase)—a nucleic acid length unit consisting of 1,000 nucleotides, also abbreviated as kbp for kilobase pairs (DNA).

Library—a set of cloned DNA fragments that contain the entire genome of an organism.

Linkage—the property of genes that are physically close to each other on a chromosome, so that they are frequently inherited together.

mRNA (messenger RNA)—RNA, transcribed from DNA, that is the template for amino acid sequence during translation.

mtDNA (mitochondrial DNA)—a small, circular, double-stranded DNA that is the mitochondrial genome. Certain regions of the mtDNA sequence are subject to rapid evolutionary change. The mtDNA is present in many copies per cell, is maternally inherited, and is useful to study maternal lineages.

Nucleotides—the monomer units that together form DNA and RNA, consisting of a purine or pyrimidine base, a pentose sugar, and a phosphoric acid group.

Oligonucleotide—a linear sequence of up to 20 nucleotides joined by phosphodiester bonds.

Plasmid—autonomous, self-replicating extrachromosomal circular DNA. Plasmids engineered as vectors for cloned DNA allow replication of cloned DNA in microorganisms (often *E. coli*). Plasmid vectors typically accept relatively small pieces of foreign DNA, usually less than 15 **kb.**

Primers—single-stranded oligonucleotides with nucleotide sequence identical to that in a target DNA. If the target DNA is denatured to a single-stranded state and then cooled in the presence of the primers, the primers will bind to the complementary (or matching) sequence on the target DNA, forming a small section of double-

stranded DNA with the target. The small, double-stranded area allows DNA polymerase to initiate extension along the remaining single-stranded section of the target, in effect creating a new matching DNA strand (see also DNA polymerase).

Probe—a labeled biochemical marker (e.g., RNA, DNA, or antibody) used to identify the presence of a specific DNA sequence, gene product, or protein because the marker binds to target molecules. The label, commonly a chemiluminescent or radioactive molecule, allows detection and location of the molecule of interest.

Restriction endonuclease (or restriction enzyme)—an enzyme that cleaves DNA molecules at recognition sites, usually defined by a sequence of four or six nucleotides in the DNA strand. The names of restriction enzymes, such as Eco RI, indicate the organism from which the enzyme was discovered (*E. coli*) and the order of its discovery (Eco RI was the first such enzyme discovered from *E. coli*).

Restriction map—a diagram portraying the linear order of restriction endonuclease recognition sites on a DNA strand.

Restriction patterns—After target DNA has been cleaved into fragments by restriction endonucleases, it is electrophoresed on a gel, separating resulting fragments by size. Fragment size reflects the distance between restriction endonuclease recognition sites in the target DNA. The number of fragments obtained is a function of the number of restriction endonuclease recognition sites in the target DNA. The restriction pattern is defined by the number, order, and size of the fragments generated by cleaving the target DNA.

RFLP (restriction fragment length polymorphism)—variable length DNA fragments generated by the action of restriction endonucleases (enzymes) on intact DNA. The fragments differ in size because mutations have created or abolished nucleotides that are part of the endonuclease recognition sequence, or because insertion, deletions, or other DNA rearrangements distinguish the two genomes.

rDNA (ribosomal DNA)—DNA that, through transcription, gives rise to ribosomal RNAs. Specific rRNA molecules combine with ribosomal protein to form ribosomes. Ribosomes are universally present in organisms, so comparisons across widely divergent groups are possible.

Ribozymes—RNA molecules that have enzymatic activity, allowing them to break and form covalent bonds.

Sequencing, DNA—determining the linear order of nucleotides along a particular DNA strand.

Southern blotting—a technique developed by E. M. Southern that involves the capillary transfer of DNA fragments resolved on a gel to a nitrocellulose filter or membrane. The DNA on the nitrocellulose filter is subsequently probed with a labeled (chemiluminescent or radioactive) complementary nucleic acid fragment. If the probe shares sequence homology with any of the fragments on the nitrocellulose it will, under the proper conditions, bind to them. The position of fragments to which the probe is bound can be identified by autoradiography. **Dot blot** procedures may be based on the Southern blot technique.

Vector—a DNA molecule engineered to allow insertion of foreign DNA (a clone) so that the foreign DNA is replicated each time the vector DNA undergoes normal replication.

YAC (yeast artificial chromosomes)—vectors replicated by yeast that allow cloning of large inserts of foreign DNA, up to 1,000 kb. The YAC is constructed with all the natural elements of a yeast chromosome.