Zeroing In on Genes

ALMOST every cell in the human body contains the same set of genes. But not all of the genes are used, or expressed, by those cells. For example, some processes that are particular to cells in the liver are completely unused in brain cells. Ever since genomic research began, scientists have been searching the tangle of DNA for the expressed genes, the ones that really matter.

If one thinks of the nucleus of a cell as a library, then the chromosomes in the cell are bookshelves and the genes are the books on each shelf. Almost every cell in an organism contains the same libraries and the same sets of books. The books represent all of the information (the DNA) that every cell in the body needs so that it can grow and carry out its various functions. Two challenges complicate the process of locating our genes: Not all of the genes are expressed in any one tissue, and less than 10 percent of our DNA is actually used to make genes. Only occasional passages in the library's written material are important.

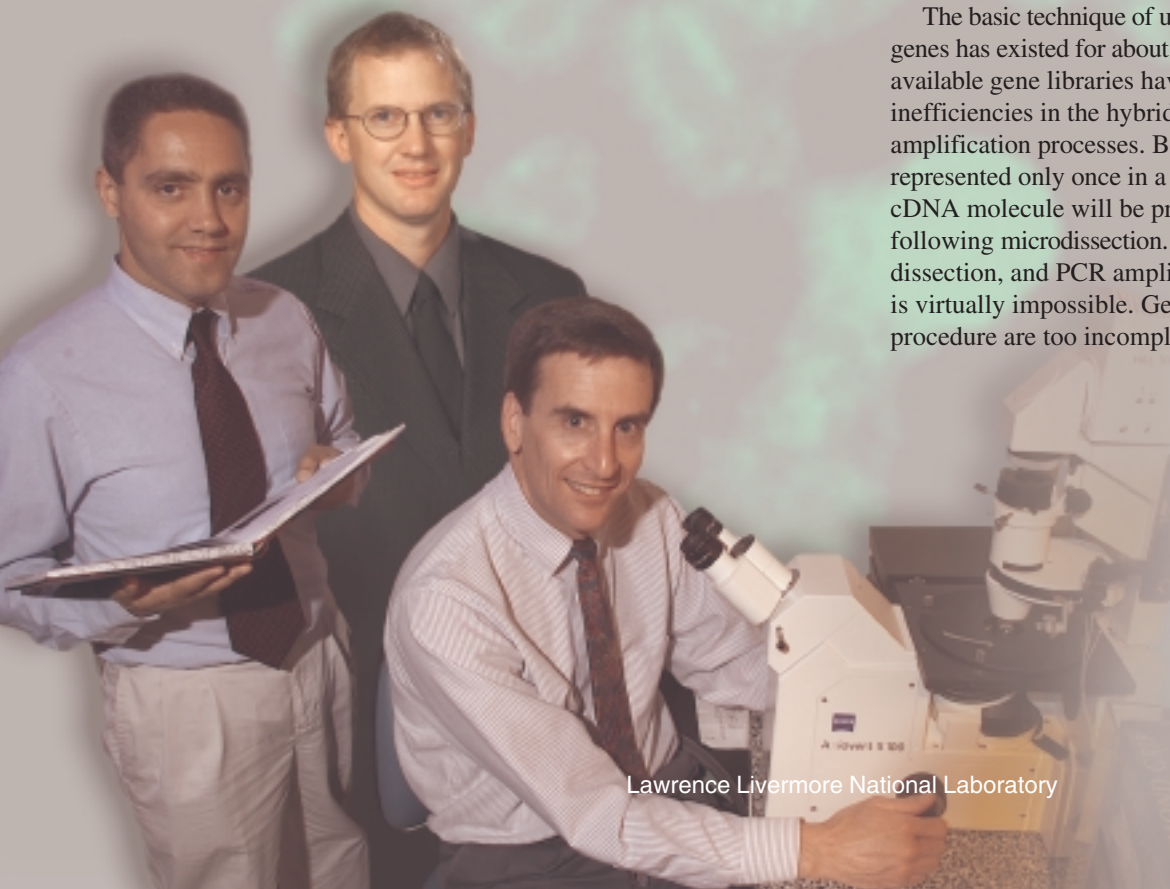
A team at Livermore led by molecular biologist Allen Christian has developed Gene Recovery Microdissection (GRM), a process that can weed out the unexpressed genetic material from a piece of DNA. With GRM, scientists can isolate all of the genes in a chromosomal region that are being used by a specific tissue at any point in time. GRM can be used for any plant or animal species. A variant of this method can also be used to clone all of the DNA of any organism, including bacteria, even those that cannot be cultured.

"It's not always necessary to sequence the entire genome of a species to locate its gene," says Christian. "With GRM, we can focus on particular regions of a genome that are of interest."

Amplification Twice Does the Trick

The product of gene expression is messenger RNA (ribonucleic acid), or mRNA. Typically, before work begins to isolate expressed genes, the mRNA molecules are converted into more stable complementary DNA molecules called cDNA, which has exactly the same sequence as the mRNA but is easier to handle in the laboratory. Then the cDNA is combined on a microscope slide with chromosomes. The cDNA molecules hybridize to the chromosome regions corresponding to the genes of which their parent mRNA is a product. Using tiny glass needles and microdissection, scientists can isolate regions of the chromosomes of interest and, with them, the hybridized cDNA molecules. Finally, amplification by polymerase chain reaction (PCR) is used to produce many copies of the molecules in preparation for DNA sequencing.

The basic technique of using microdissection to isolate genes has existed for about five years. But no commercially available gene libraries have been generated because of inefficiencies in the hybridization and subsequent PCR amplification processes. Because genes are typically represented only once in a chromosome, a maximum of one cDNA molecule will be present for each expressed gene following microdissection. Successful hybridization, dissection, and PCR amplification of a single molecule is virtually impossible. Gene libraries made with this procedure are too incomplete to be useful.



Lawrence Livermore National Laboratory

Developers of the award-winning Gene Recovery Microdissection process are (left to right) Matthew Coleman, Allen Christian, and James Tucker of Livermore's Biology and Biotechnology Research Program Directorate.

Livermore's GRM process overcomes this inefficiency by combining cytogenetics and genomics with chromosome microdissection. CRM increases both the number of targets available for cDNA hybridization and the total number of cDNA molecules in each region following hybridization.

The trick is to perform PCR amplification in situ, on the slide rather than in a tube, which is the conventional means. And it occurs twice. First, prior to hybridization, random-primed PCR of the chromosomes on the slide produces many copies of the target DNA, significantly improving the chances of cDNA hybridization. Second, following the hybridization, another PCR amplification using primers specific for the ends of the cDNA molecules increases the numbers of bound cDNA molecules. Instead of isolating just one cDNA molecule per expressed gene in a region, the GRM process recovers hundreds or even thousands of cDNA molecules. This simple step makes possible the production of highly useful chromosome-region-specific libraries.

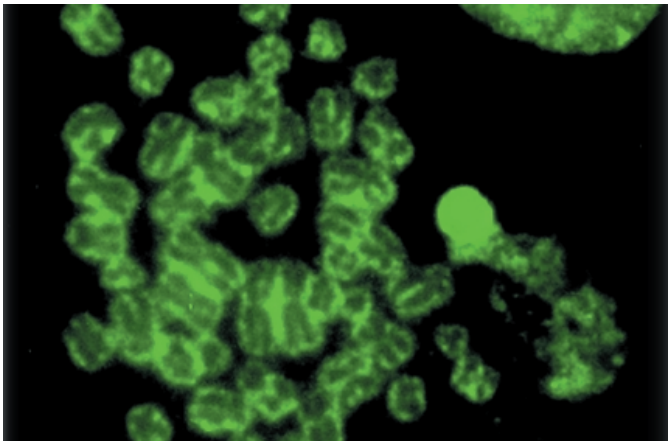
GRM has other advantages. While cells generally contain only one or two copies of a gene, some genes make thousands of copies of mRNA and others make only a few copies. Finding mRNA molecules with a low number of copies amid the "noise" of the more numerous gene products can be difficult with conventional methods of making cDNA libraries. But the hybridization step in GRM results in a balanced library in which mRNA molecules with high and low numbers of copies are equally represented.

Several companies offer processes that provide partial information about gene expression and genomic location. But no other single technique identifies both known and unknown expressed genes and determines the part of the genome that regulates their expression. GRM makes possible in one process what multiple processes could previously handle only in part, and it does so cost effectively. Current estimates are that the costs associated with GRM will be substantially less than those of traditional methods. The process is also significantly faster.

Benefits Abound

GRM was invented to allow researchers to identify cancer genes in chromosomal regions for which no genomic information existed. Initially, these were regions for which scientists had good evidence of their importance in rat mammary cancer but almost no other knowledge. To identify the genes expressed in these regions, researchers needed a quick, simple, inexpensive, and reliable method of identifying and characterizing both new and previously known genes in chromosomes.

GRM focuses on data that current genomic sequencing efforts do not provide, namely, information concerning the expression of genes in specific regions of abnormal cells, such as those found in cancerous tissue. "We are using GRM to learn which genes are expressed in certain parts of chromosomes in cancer cells," says Christian. "We can then compare our data with data from the Human Genome Project and learn how these particular cancer cells differ from normal cells."



Chromosomes amplified by Gene Recovery Microdissection. Amplification is by polymerase chain reaction and produces many copies of stable complementary DNA molecules in preparation for sequencing.

GRM will be used to generate chromosome-specific and chromosome-region-specific libraries of genes that are expressed for any tissue, normal or diseased, of any organism that can have its chromosomes spread on a microscope slide. Once these libraries have been produced, they can easily be placed on microarrays and made available to other investigators for more detailed analyses, including gene expression studies. GRM can thus be used to create a systematic approach to identifying genes expressed in virtually every species of interest to humans. This capability opens the door to sequencing many plant and animal species that might otherwise be ignored because of the prohibitive cost of genomic analysis. Agriculture, environmental sciences, and veterinary medicine will all benefit.

GRM technology provides the preliminary step toward a full genomic analysis of an organism, allowing time and money to be saved during the full analysis. This invention will enable scientists to identify genes that are expressed after exposure to drugs, environmental chemicals, or radiation. Toxicologists can study the reactions of cells and organisms to chemical and radiation exposure, furthering basic understanding of the molecular mechanisms involved in responses to adverse environments. Similarly, the pharmaceutical industry will be able to decipher biological responses to drugs.

—Katie Walter

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