Sending Up Signals for Genetic Variation

THE Human Genome Project, the international research program to map and sequence all the human genes, is an immense scientific effort that has made demands on biological research techniques and led to new biological tools. Among the many developments resulting from genomic research is one by a Livermore team that can find minute changes in the DNA of individual cells and thereby significantly improve the detection of cancer and other diseases.

The new technique, called in situ rolling circle amplification (IRCA), is a fast and inexpensive method to precisely locate a damaged or abnormal gene that indicates the presence of or tendency toward a particular disease. IRCA can find a single cell containing an abnormal gene from among thousands of cells, making the technique ideal for tissue biopsies. The process is so sensitive that it can detect a mutation in a single DNA base, the smallest unit of genetic information. (An average human cell contains about 6.5 billion DNA bases.)

The technique involves locating the gene of interest in intact cells—in which fluorescent molecules have been incorporated—and massively amplifying (duplicating) critical sections of DNA so a fluorescent signal can be detected. No other method is available that can detect a single DNA or RNA base change within a cell or tissue.

"IRCA is a way of putting our newly gained knowledge of the human genome to beneficial use," says team leader Allen Christian, a molecular biologist and chemical engineer. The discovery, he says, moves the research findings of the Human Genome Project from the laboratory to the clinic. "The genome project has given us the sequences of all the human genes. Our job as scientists is to apply that knowledge. We know that certain DNA sequence variations signal a diseased state, so we can use that information to diagnose disease at the earliest stages, when treatment is most effective."

The Livermore research team, which worked for about a year on the process, included biologist Jim Tucker and technicians Melissa Pattee and Christina Attix. (Christian and Tucker were part of a Livermore team that won an R&D 100 Award last year for gene recovery microdissection, a process that identifies expressed genes—that is, genes that have been turned on—from a specific chromosome region.)

The Livermore breakthrough is a significant extension of rolling circle amplification (RCA), which was developed at Yale University in the mid-1990s. RCA is limited to identifying DNA that has been extracted from a cell. In contrast, the Livermore technique works inside cells, thereby preserving the chemical environment of the cell and its neighbors. In addition, IRCA provides answers in a couple of hours, compared to a wait of several days required with tests using traditional methods. The technique can also be used to detect and measure messenger RNA in single cells, something that could not be done previously. (Genes produce proteins with the aid of messenger RNA.)

The team that developed the in situ rolling circle amplification technique. From left, Allen Christian, Melissa Pattee, Jim Tucker, and Cristina Attix.

Lawrence Livermore National Laboratory

Synthetic Probes Start the Process

The technique starts with the laboratory production of linear probes (or stretches) of DNA encompassing about 100 bases. The DNA has been treated to remove one of its double strands. The probes are applied to target DNA under study, which also have been made single-stranded. Because DNA prefers to be double-stranded, the probe will seek out a target whose DNA sequence is a counterpart to that of its own.

In the cell nucleus, the linear probe's two ends attach through hydrogen bonds to the target DNA and in the process wrap around that DNA to form a circle. An enzyme called a ligase then "padlocks" the circular probe onto the target DNA by forming chemical bonds that are much firmer than hydrogen bonds. In this way, the probe is prevented from detaching from the target.

Typically, two probes are applied to the target DNA: a normal sequence probe and a mutant sequence probe. The normal probe can only attach to the normal DNA sequence, and the mutant probe can only attach to a mutant sequence.

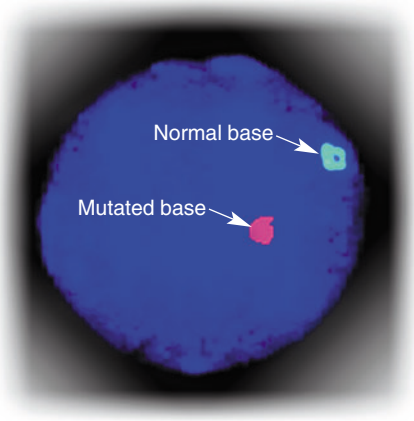
Each probe contains a DNA sequence that allows a short piece of DNA, called a primer, to initiate a reaction that quickly and repeatedly replicates the probe. The duplicating reaction is catalyzed by a polymerase enzyme, which rolls out hundreds or thousands of linear copies of the circular probe's DNA. The single strands of targeted DNA make it easier for the polymerase to make copies of the probe.

Every time the probe is replicated, a binding site for a fluorescently labeled molecule (or beacon) is created. Within a few seconds, enough beacon sites are created to allow the fluorescent signal to be seen under a microscope and permit a normal base to be distinguished from an abnormal base. "We're in essence sending up signal balloons that help us to detect the products of the polymerase reaction," says Christian.

The match between a normal probe and target produces a green signal after amplification, whereas a match between a mutant probe and target produces a red signal. If the probe and the target do not match, there is no signal because the ligase will not work.

Numerous Applications

IRCA has numerous medical applications, especially in the diagnosis and treatment of diseases that have genetic markers. Christian expects that one of the technique's first routine clinical uses will be as a fast, inexpensive assay for the presence of mutations that are relevant to a particular cancer. "IRCA will allow physicians and medical researchers to identify and localize genes that are known to be or strongly suspected of being responsible for causing certain cancers," says Christian. The technique will also help physicians to choose and customize cancer therapies for their patients and monitor the effectiveness of therapies.



The in situ rolling circle amplification technique reveals a mutated tp53 gene in a human lymphoblastoid cell by coloring it red, whereas a normal gene is colored green.

Christian notes that many cancer diagnostic procedures currently in use involve the same dyes and stains that were first discovered more than 100 years ago. These dyes and stains cannot detect the subtle genetic changes that are believed to occur when tissue first becomes cancerous.

On a different application, IRCA could be used to identify the strain of bacteria infecting a person. This capability would enable physicians to select the most effective antibiotics. Similar approaches may work for detecting viruses ranging from the common cold to hepatitis, herpes, and HIV.

IRCA also has applications in agriculture, toxicology, pharmacology, and environmental science. The technique will assist scientists in rapidly identifying which strains of a plant, tree, or vegetable have desired genetic characteristics. In this way, IRCA has the potential to improve the quantity and quality of food. In basic cell research, the advance could help to determine the genetic composition of bacterial, plant, and human cells. For pharmacology, the technique will provide an important tool to test promising new drugs and measure cells' responses to them.

The technique may also have an important role to play in fighting bioterrorism. Portable detectors using IRCA may offer advantages over units using the polymerase chain reaction technology to amplify short stretches of DNA or RNA and thereby identify a potential bioagent.

Christian reports that the research team has been flooded with calls and e-mails from researchers across the country since the procedure was first described in a paper published in *Proceedings of the National Academy of Sciences* in 2001. The Laboratory is currently negotiating with companies to license the process.

Clearly, IRCA stands to improve human health and advance a large number of disciplines.

—Arnie Heller

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