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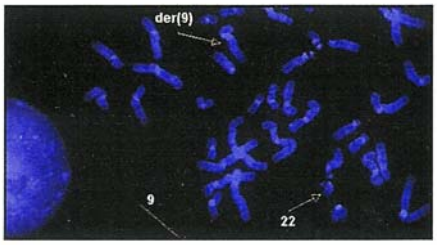
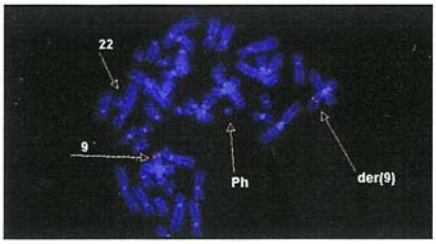
Columns





Single-copy FISH probes the genome

by Dr. Ricki A. Lewis
Contributing Editor

SUMMARY

By focusing on DNA sequences that are present in the human genome in single copies, a new variation on FISH provides high-resolution, specific views of the genetic basis of birth defects and cancer.

The wedding of fluorescence in situ hybridization (FISH) to human genome sequence information is taking an already targeted technology to a new level of specificity. The inventors of single-copy FISH (scFISH), molecular geneticist Peter K. Rogan and cytogeneticist Joan H. Knoll at Children's Mercy Hospital and Clinics, University of Missouri-Kansas City School of Medicine, unveiled the tool at the XIX International Congress of Genetics in July in Melbourne, Australia.

"With the human genome sequence available, Pete and I pooled our expertise to develop FISH probes directly from the genome sequence," Knoll said. They have already demonstrated many eclectic clinical applications.

Visualizing human chromosomes has come a very long way since 1923 when University of Texas geneticist Theophilus S. Painter sketched the first crude views. When FISH made its debut in 1988 as a fluorescent version of in situ hybridization using radioactive DNA probes, it was a vast improvement and soon became an adjunct to routine cytogenetics testing, which characterizes chromosomes at the band (or multigene) level. In contrast, scFISH detects small chromosomal segments that span a few genes to parts of a gene.

One limitation of commercially available FISH probes is that most are considerably larger than their intended targets. Such a probe might cover 100 to 180 kilobases (100,000 to 300,000 DNA bases), when only a few kilobases complement the gene or genes that affect health. "We realized that we could find intervals of single-copy DNA of about two kilobases and synthesize them in vitro, prepare and hybridize these probes. Single-copy probes are much smaller and much more densely represented on a chromosome. Therefore, we can look at much smaller lesions," Rogan explained.

Another advantage of scFISH: It can probe rare conditions. "The ones that are clinically available detect relatively common abnormalities," Knoll said. "I direct a clinical cytogenetics laboratory, and there is an insufficient variety of probes to meet patient needs." Even commonly encountered chromosomal anomalies may harbor nuances that are beneath the radar of conventional FISH. These can yield their secrets to the single-copy approach.

Making scFISH probes

Designing FISH probes that home in on specific gene parts is more computational than cytogenetic. Software is used to select DNA sequences that appear in only one place among the chromosomes and to design PCR primers to amplify the region from genomic DNA. (The probes also may be synthesized directly.) Rogan and Knoll select part of the human genome sequence and identify the repetitive sequences, which must be eliminated to maintain the probe's ability to hybridize to the specific chromosomal interval and not to other genomic addresses that harbor the same repeats. The software then determines the lengths and locations of the remaining single-copy intervals. These are further analyzed to eliminate probes containing sequences very similar to others, such as pseudogenes or members of gene families.

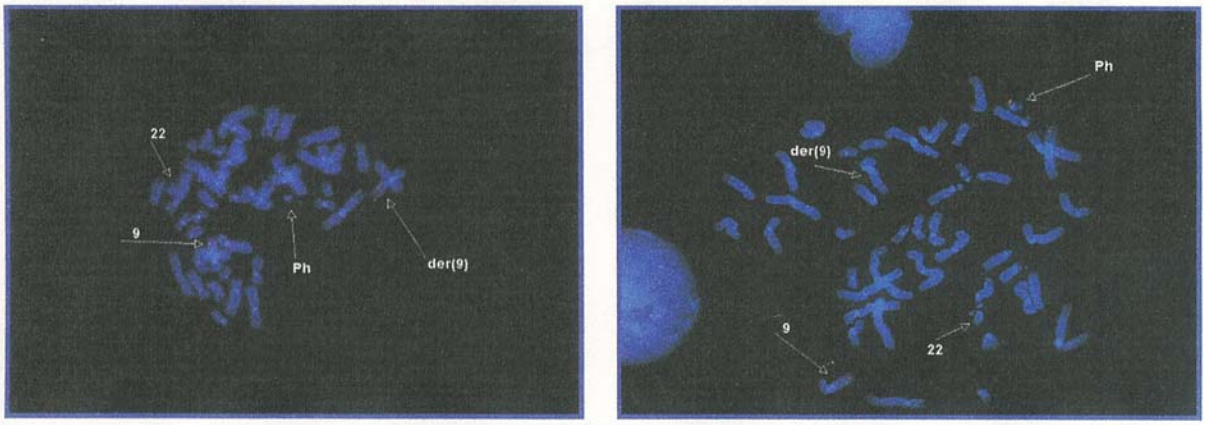
The scientists modify amplified sequences with digoxigenin- or biotin-conjugated deoxyuridine triphosphates, then hybridize them to chromosomal DNA labeled with digoxigenin or biotin and detect them with fluorescein or rhodamine. They visualize the probe sequences with an epifluoresence microscope equipped with a CCD camera and CytoVision ChromoFluor software from Applied Imaging Corp. of Santa Clara, Calif.

The researchers scrutinize cells that are in metaphase when chromosomes are condensed and visible, so that the fluorescent labels clearly mark their chromosomal locations. "For the applications we are developing, it is not sufficient to simply count spots. We must know the genomic context of the probe on the derivative chromosome," Rogan said. Probe design takes about a half hour on a Unix workstation; synthesis and hybridization are complete generally in less than a week. Developing conventional FISH probes can take much longer.

Applying arrays of single-copy probes that hybridize in an overlapping fashion can enhance the signal. "This also can precisely localize a chromosomal breakpoint based on which chromosome harbors the hybridized signals, because the probes are very small and are derived directly from the genome sequence," Rogan said. For example, in one form of acute myelogenous leukemia, a chromosome segment inverts, separating adjacent probes. A conventional FISH probe that encompassed much more than just the inversion site would determine only the general region containing the chromosomal break. The small size of the probes speeds the determination of the breakpoint analysis, he added.

In a paper in the June 2001 issue of *Genome Research*, the scientists described their method and validation procedures. They created test probes to sites on three chromosomes that, when deleted, cause well-characterized conditions: "monosomy 1p36 syndrome" on chromosome 1; Prader-Willi and Angelman syndromes arising from parent-of-origin specific deletions of the same region of chromosome 15; and DiGeorge syndrome, which is caused by deletion of part of chromosome 22. Resolution was better than with conventional FISH probes.

The researchers also examined the trio



Chronic myelogenous leukemia is linked to a gene called ABL on chromosome 9, moving, or translocating, near a gene called BCR on chromosome 22. These fused genes are on the very small Philadelphia (Ph) chromosome. Single-copy FISH probes that correspond to portions of the ABL gene helped researchers to zero in on the point where the two gene parts meet. The image on the left shows probe 16, which corresponds to one end of the ABL gene. It does not move to the Philadelphia chromosome, as indicated by the fluorescence (red) on chromosome 9 and the chromosome derived from chromosome 9 [der(9)]. In the right panel, the two probes used correspond to the middle part of the gene and are translocated to the Philadelphia chromosome, where they flash (red). Courtesy of Joan H. Knoll and Peter K. Rogan.

of probes for telltale signs of the uniqueness of single-copy sequences: higher than average GC content, presence of introns and exons, and comparison to the human genome sequence. Then they applied the technique to two chromosomal extremes to see if it is effective anywhere in the genome. ScFISH did well on both gene-poor chromosome 21 and gene-packed chromosome 22. One or more single-copy probes of two kilobases or greater can be found in most genomic intervals of 100 to 150 kilobases.

ScFISH frontiers

Analysis of the human genome sequence is providing a seemingly unending list of questions that scFISH can answer. In a paper published in the *American Journal of Medical Genetics* online June 12, 2003, Rogan and Knoll reported the successful application of the technique to 74 additional chromosomal addresses, with intriguing clinical revelations.

One example is Williams-Beurer syndrome. Affected individuals have an elfin-like face, elevated blood calcium, developmental delay, learning disabilities, and narrowed heart and lung arteries. Two overlapping scFISH probes of 3049 and 2264 bases homed in on the gene LIMK1, which has been implicated in the cognitive deficit in this disorder. The current commercially available 180-kilobase probe includes that gene and the elastin gene, potentially complicating the diagnostic picture.

Single-copy probes can also see what conventional FISH probes can't. Smith-Magenis syndrome, for example, is nearly always associated with a large deletion in chromosome 17. Affected individuals exhibit short digits, small facial features, hoarseness, speech delay and self-destructive behavior. But scFISH adds diagnostic precision for children who have the symptoms but lack the characteristic huge deletion — their deletions are just too small for traditional FISH to pick up.

A third application of scFISH took center stage in the work that the researchers presented in Melbourne: a new view of the genetic abnormality found in chronic myelogenous leukemia. This is the cancer that recently made medical history because of the spectacular success of treatment with the drug Gleevec.

In this form of leukemia, chromosomes 9 and 22 exchange parts, generating an overactive version of a gene that encodes the ABL1 tyrosine kinase, an enzyme that normally receives signals for cell division. Gleevec nestles into the pocket on the enzyme that binds ATP, which is necessary to fuel division. But initial success with the drug was quickly followed by reports of patients who are resistant to it. Because most have additional mutations, either in or near the gene, Knoll and Rogan turned to scFISH to identify the affected chromosomes. "We used probes for chromosome 9 genes distributed throughout this 400-kilobase region to look for smaller rearrangements and deletions," Knoll related.

By overlapping probes that hover around the breakpoint, the researchers confirmed that some of the patients had a second glitch, a very small deletion. Conventional FISH, with its larger probes, might have missed this subtle yet clinically telling distinction, Rogan said.

With the continuing annotation of the human genome, single-copy FISH is certain to foster many eclectic applications that will fit in beautifully with the coming personalization of medicine. The technique may fill a key niche in human genetics as a bridge between the gene and chromosomal abnormalities found in congenital disorders and cancer. The researchers expect to receive the first of several pending patents this fall. □