on, Applied Biosystems turned down both groups, although Mathies says he is now in negotiations with a biotech company aimed at licensing his technology. So for the moment, the intrepid adventurers of the human genome will have to keep waiting for the tools that will make their dream a practical possibility.

The Dyer's Hand

Although a child may love his yo-yo, and the Wizard of Oz's Dorothy surely loved Toto, you wouldn't think those affections would have any particular relevance to molecular biology. But molecular biologists are sure to love a new pair of creatures with the whimsical names of YOYO and TOTO. At the Science Innovation conference, researchers reported development of these two new DNA dyes, which, cute names aside, have achieved an important technical milestone: They can detect minute amounts of double-stranded DNA—as small, in fact, as radioactive probes—but without the danger or mess inherent in radioactivity. As a result, they have the potential to make significant contributions to genome mapping and DNA fingerprinting, among other applications.

YOYO and TOTO have "the charm of simplicity," says Alex Glazer, who along with graduate student Hays Rye and others developed these dyes at the University of California, Berkeley, in collaboration with Stephen Yue and Richard Haugland at Molecular Probes Inc.

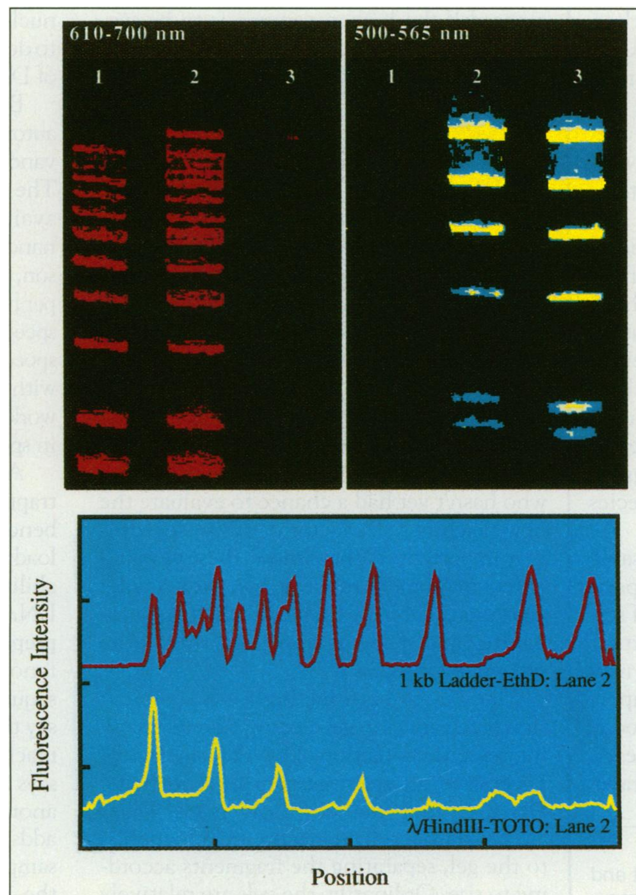
And the inventors aren't the only ones touting the dyes. "I really see the future as being very bright for these molecules," says Jasper Rine, head of the Department of Energy's Human Genome Project at the Lawrence Berkeley Laboratory. "As a replacement for radioactive detection of small quantities of DNA, they have the capacity to make major improvements in genome research," he says.

The reason for the enthusiasm is that for years researchers have been looking for alternatives to radioactive probes for detecting DNA. Although radioactivity is currently an indispensable tool because of its unsurpassed ability to label and detect small amounts of material, it's a four-letter word to biotech entrepreneurs and managers of big efforts such as the Human Genome Project. Radioactive methods are dangerous in the hands of semiskilled workers, and they are hard to automate; they're expensive, awkward, and time-consuming; finally, radioactivity is highly non-politically correct, environmentally speaking. In search of replacements, scientists have used everything from fluorescent dyes to chemical reactions that give off light to the firefly's light-emitting molecules. Yet these alternatives either lack sensitivity or are cumbersome. "I've been attending nonisotopic meetings for a dozen years," says Irena Bronstein of Tropix Inc., who organized a session on alternatives to radioactivity at the innovation conference, "but radioactivity is still around."

Now come Glazer's dyes, which have the double charm of being easy to use and about 500 times more sensitive than often-used dyes like ethidium bromide. There is a catch, however: Using a simple light-box to excite the dyes yields only a 25-fold improvement over regular dyes, Glazer says. To get the full 500-fold improvement requires some moderately fancy equipment, including a laser and sensitive detectors. For that, Glazer turned to Rich Mathies, a spectroscopist in Berkeley's chemistry department. The result? The ability to detect a mere 4 picograms of DNA in a single gel band "as good or better than [using] radioactivity," says Mathies.

Behind that achievement lies a simple conceptual strategy: If a single DNA dye is good, a dimer of that dye should be better. In the 1970s Jean-Bernard Le Pecque and his colleagues took two ethidium bromide molecules, stuck them together with a short linker, and made "ethidium homodimer." The result was a vast improvement over conventional dyes. In the new round of work, Glazer and co-workers adopted the same strategy but started with even better dyes, oxazole orange (YO) and thiazole orange (TO), to make the dimers YOYO and TOTO. The results are dyes that absorb light unusually well, stick to DNA like glue, and fluoresce when bound to DNA but have virtually no fluorescence when not bound to DNA. All of which means plenty of signal and little background.

That's good news for biologists who work with small amounts of DNA. At LBL's genome center, staff scientist Alla Lishanskaya is already using the dyes in a new procedure to test for carriers of genetic diseases. She compares a patient's DNA with a complementary DNA strand of normal sequence by hybridizing them and looking for mismatches, which would indicate a mutation. But she has to work with small amounts of a patient's DNA—because amplifying the DNA by using many cycles of the polymerase chain reaction would introduce sequence errors in the DNA. Glazer's dyes let her use less DNA, and the result is fewer false positives.



Living color. Having two ultrasensitive fluorescence dyes makes it possible to measure the length of DNA fragments with considerable consistency. The upper panels show two fluorescent images of a single gel, seen through two different colored filters. The images show that two samples labeled with different dyes (lanes 1 and 3) can be mixed together (lane 2) yet detected separately, improving the accuracy of length comparisons. The lower panel shows the peaks of fluorescence intensity.

And that's not all. Glazer points out that the new dyes could have important benefits for DNA forensics and genome mapping. In both fields it's important to measure the length of a specific DNA fragment by running it through a gel, which is often not easy to do accurately. Indeed, variations from gel to gel and from lab to lab in determining DNA fragment lengths have been one source of the controversy over DNA fingerprinting. Now, by using two of Glazer's dyes simultaneously, labs may be able to achieve greater accuracy than with current techniques—though Glazer concedes he's just started to examine the issue in depth. Having two supersensitive dyes makes it possible to run both a "control" DNA fragment of known length acting as a yardstick, labeled with one dye, and an unknown sample, labeled with the second dye. Such a procedure, not feasible with radioactivity, greatly improves precision and reproducibility. And that, along with the other potential uses for these new dyes, is enough to make many people involved in DNA detection light up.

–Paul Selvin

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