BIO/TECHNOLOGY SINGAPORE CONFERENCE

AMPLIFIED GENES AND FRAME-SHIFT MUTATIONS

SINGAPORE—New gene-amplification technology could make it possible to detect DNA needles in heterogeneous sample haystacks. Speaking here in October, Henry Erlich (Cetus Corp., Emeryville, CA) presented some new modifications—and new applications—of polymerase chain reaction (PCR) technology for amplifying specific DNA sequences more than a million-fold. Erlich's platform was "The Molecular Biology of Human Disease: An Asian Perspective," a symposium sponsored by *Bio/Technology*, the Science Council of Singapore, and the National University of Singapore.

Polymerase chain reactions use two oligonucleotide primers flanking a target DNA sequence. These primers initiate repeated cycles of annealing, extension, denaturation, and re-annealing, achieving what amounts to a cell-free molecular cloning of a well-defined target polynucleotide. Recently, Erlich's team incorporated a thermostable DNA polymerase from *Thermus aquaticus*, a hot-spring alga, that permits continuous operation without adding fresh enzyme after each denaturation. The hardy polymerase also helps increase the technique's sensitivity and yield, and allows amplification of longer target DNA chains. It also makes the process easier to automate: Perkin-Elmer Cetus Instruments should begin marketing a PCR instrument soon, Erlich said.

The technique has applications where small amounts of DNA are critically important—in forensic analysis, for example, or in detecting integrated human immunodeficiency virus (HIV). Erlich showed that a single beta-globin template could be readily identified from a mixture of more than a million other cells after 60 PCR cycles. With a few more cycles, the technique could begin to detect a single target against a background of some ten-million cells, he said. Current techniques detect HIV provirus by *in situ* hybridization; they are several orders of magnitude less sensitive, and are not yet automated. Thus, an affordable PCR diagnostic kit might sharpen the epidemiological and diagnostic pictures, by distinguishing between those who carry antibodies to HIV and those who carry HIV provirus itself.

Another researcher, Anthony P. Monaco of the Boston Children's Hospital, discussed other research with important diagnostic implications. Monaco is a member of the group, led by Louis Kunkel, that first identified the Duchenne muscular dystrophy (DMD) gene—a sprawling, huge 14 kilobase gene spread very thin over some 2 million bases on the X chromosome. The gene is chopped into some 120 exons, averaging just 150 bases long, separated by introns of about 16 kb. The gene is thus very vulnerable to spontaneous mutation. Monaco's own research focuses on the differences between the severe DMD, which usually kills the victim by age 30, and the much milder Becker muscular dystrophy (BMD). Both appear to be deletion mutations—indeed, while one deletion in a given area may produce DMD, a different deletion affecting the same region will cause BMD. Monaco's answer to that puzzle: While some deletions produce frame-shift errors, others do not. Patients with frame-shifted genes produce no functional protein and develop DMD. Those in whom the frame is unshifted, however, produce some semi-functional protein and develop BMD.

—Harvey Bialy and Douglas McCormick

BIOTECH USA

MORE APPLICATIONS SEEN FOR IMMUNOASSAYS

SANTA CLARA, Calif.—Big Brother Biotech may soon be watching you more closely than ever before, whether it's testing the quality of fast-food hamburgers, analyzing blood samples to determine exactly when drugs were taken, or performing myriad other highly specific assays. In fact, using monoclonal antibodies to render novel diagnoses was a theme that echoed through several different meeting halls at the "Biotech USA" conference and exhibition held here in early November.

David Brandon, a research chemist at the U.S. Department of Agriculture's Western Regional Research Center (Albany, CA), stressed that monoclonals will play an increasing role in measuring beneficial or adverse changes that food proteins undergo during processing. The tests could, for example, quantitate potentially allergenic or dangerous components, such as wheat gluten (which generates mutagens when heated with carbohydrates). For applications like these, however, it may often be necessary to detect multiple epitopes because toxicity can be caused by several compounds.

Immunoassays can also be developed to scan for adulteration of processed foods, including the presence of horse meat or soy filler in hamburgers. In this case, the need would be to produce antibodies against stable epitopes that are not altered during cooking. Additionally, immunoassays could be used to measure a protein's activity, such as the protease inhibitory activity of soy products. Here, an antibody that specifically reacts with the enzyme's active site is required.

The unique precision of monoclonals also promises—or threatens—to bring a new dimension to assaying for drug abuse. Crucial in developing such tests, according to Carol Whisnant, a research immunologist at Research Triangle Institute (Research Triangle Park, NC), is to decide whether the antibody needs to be highly specific (and recognize just the drug itself or one of its important metabolites) or broad-based (and bind to all the related compounds). In order to generate antibodies to these small, mostly non-immunogenic molecules, scientists attach them to larger proteins such as human serum albumin. Whisnant stressed that the way in which the drug hapten is linked to the protein is key because antibodies formed will tend to be specific for the portion of the hapten *away* from the linker.

Herbert Platt, president of Genetic Diagnostics Corp. (Great Neck, NY), plans to use monoclonals in a particularly elegant way. By harnessing antibodies directed toward various cannabinoid metabolites, he believes that immunoassays will prove accurate enough to determine ratios between the various compounds. Based on the rates at which the body breaks down these chemicals, these ratios should, for the first time, allow pinpoint assessments of when the drug was actually taken.

For the immediate future, however, Genetic Diagnostics' goals are somewhat more modest: During the first quarter of 1988, the firm expects to market polyclonal tests to detect cannabinoids and cocaine, and a monoclonal-based assay for benzodiazepines. Also under development are immunoassays for LSD, morphine, and opiates. **—Arthur Klausner**