Electrochemical "SNP-its"

Single nucleotide polymorphisms (SNPs) are highly conserved single base changes that occur throughout the genome, making them ideal markers for genetic variability. Several distinct SNPs have been linked to genetic diseases so far, and much effort is being invested in developing efficient, cost-effective, and high-throughput methods to screen large populations for their SNP profile. Direct genome sequencing would be ideal, but the method is time-consuming and expensive. Similarly, DNA-chip-based methods are extremely high-throughput but can be expensive and are difficult to adjust when looking for new markers.

Recently, Sara Brazill and Werner Kuhr of the University of California, Riverside, developed a primer extension assay that seems to fit the bill (*Anal. Chem.* **2002,** *74* (14), 3421–3428). In this method, a primer is designed such that its 3′ end binds adjacent to the SNP of interest. A DNA polymerase that incorporates the appropriate dideoxynucleotide triphosphate (ddNTP) then extends the primer by one nucleotide. The ddNTP prevents the chain from extending beyond the SNP site. Thus, a 20-base primer will be extended to 21 bases only when reacted with the appropriate ddNTP. The two primers are then separated by capillary gel electrophoresis (CGE) and their concentrations determined.

Although such single base extension methods have been used extensively, most previous work has relied on fluorescent labeling of the primer or ddNTP for detection. Brazill and Kuhr, in contrast, labeled the primer on its 5′ end with ferrocene, which allowed them to detect the reaction products electrochemically by using sinusoidal voltammetry (SV). As the primers pass a carbon fiber electrode at the end of the capillary, they perturb the sinusoidal voltage, and the degree of perturbation is linked to primer quantity. The sensitivity of SV—the nanomolar to picomolar range—makes it ideally suited for assay miniaturization.

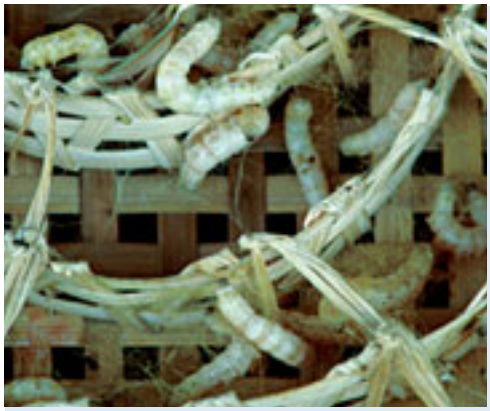
Although this method is still in the developmental stages, the researchers are confident that the coupling of single base extension with CGE and SV will provide a fast, simple, and inexpensive SNP screening technique.

—RANDALL C. WILLIS

5′-ACTCACTATAGGGCGAATTGG.....XCCCTTTAGTGAGGGTTAATT-3′ Template

3′-GGGAAATCACTCCCAATTAA–ferrocene–5′ Primer

Extension detection. A template with a "mock" SNP site labeled X was annealed to a 20-mer primer with a ferrocene acetate tag. When the SNP's complementary base is added, single base extension of the primer can be detected electrochemically.



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No silken shrouds?

According to a recent report by scientists at the Seoul National University (Korea), the "blood" of silkworms can hold off the Grim Reaper, at least in specific human cells infected with normally apoptosis-inducing vaccinia virus.

In the study, the Korean researchers harvested hemolymph, the oxygen-carrying fluid that suffuses through the open circulatory system of insects as blood, from silkworm larvae and added it to a culture medium of virus-infected cells to analyze its effect on apoptosis (*Biotechnol. Prog.* **2002,** *18* (4), 874–878).

Because DNA breakage between nucleosomes is a key indicator of apoptosis, two assays measuring breakage were undertaken: A fluorescent assay was used that marked such breakages in vivo, and a gel assay was used on extracted cellular material that demonstrated DNA cleavage by showing the production of DNA ladders. In addition, the scientists used standard viability assays to measure the percentage of cell death.

All three assays indicated that the presence of hemolymph inhibited vaccinia-virus-induced apoptosis. Significantly, bioassays showed no apparent inhibition of either virus infection or reproduction. Thus, the inhibition of apoptosis appeared to be specific to that pathway rather than due to interference with the virus itself. As a note added in proof, the researchers indicated in data not shown in their paper that hemolymph also interfered with apoptosis caused by nonviral chemical triggers, including cisplatin and actinomycin D.

The authors assume that the anti-apoptosis effect is specific and is mediated by a single product from a gene that they hope to clone one day. Although this is perhaps overspeculation based on the complex mix of materials that insect hemolymph contains, their optimism may not be wholly misplaced. Previously, these researchers isolated a nonglycosylated monomeric protein (ca. 28,000 Da) from hemolymph that inhibited apoptosis in insect cell cultures.

Although apoptosis often provides the only protection against several diseases—by killing off infected cells before pathogens can spread or tumors can grow—there are a number of degenerative diseases in which aberrantly triggered apoptosis plays a deadly role. If a natural anti-apoptosis product can be isolated from silkworm larvae hemolymph and the gene cloned, the possibilities for basic research, commercial cell production, and human therapeutics could be great.

—MARK S. LESNEY

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