

AACC MEETING

DNA PROBES REVISITED

SAN DIEGO, Calif.—Every year about this time scientists and analysts alike try to get a feel for the size of the DNA probes market. Today's fortune tellers may be a bit more reliable than their predecessors, their vision tempered by the reality of 1988's \$25-million DNA probes market and a decade's experience with the limitations of probe-based technologies. Past problems with probe sales have stemmed from regulatory delays, high production costs, problems in standardizing assay formats, and competition from immuno-based detection systems.

Skepticism in the face of soothsaying is healthy—but industry insiders aren't gun shy. Speaking at the two latest DNA probes meetings sponsored by Communitel Market Intelligence (Yorktown Heights, NY) and the American Association of Clinical Chemistry (AACC, Washington, DC), Richard Taylor, manager of applied biotechnology at Arthur D. Little (ADL, Cambridge, MA) predicts total DNA probe sales will grow to \$100 million by 1995. Participants predicted that the first automated DNA probe-based analyzer would be on the market in two to five years. And Charles Cantor, director of the Human Genome Center at Lawrence Berkeley Laboratory (Berkeley, CA), reminded the audience of market opportunities stemming from sequencing the human genome, which he estimates will happen within 15 years.

Probe cost is the limiting factor for implementing the current, polymerase chain reaction (PCR)-based strategies for the genome project, says Cantor. He thinks that chromosome walking by conventional methods or inverse PCR is the way to get the most information from DNA. Both methods rely on synthesizing new probes as you go further along the DNA, which requires synthesizing 10 percent of the three billion base pairs in the human genome. "At \$10 a base, this would cost \$3 billion just on probes," said Cantor. "The major need is getting the costs of probes down."

While it is easy to be swept away by the hype inspired by the genome project—like predictions that the face of medicine will change since disease diagnostics will be individualized—it is sobering to remember that manufacturers aren't pushing too hard to develop automated clinical DNA-based analyzers, except perhaps for infectious disease detection. "A diagnostic test without a therapeutic

doesn't really have a market," said Edward Chait, a sales manager for DuPont (Wilmington, DE).

Yet researchers are forging ahead, developing systems for nonisotopic detection, homogeneous assays, and automation. More efficient, cost-effective diagnostics still are needed in the clinical setting.

ADL's Taylor was particularly fond of developments in dual-probe homogeneous liquid assays as a step towards eventual automation—even though its sensitivity is only 500,000 targets. In this type of system, two probes that hybridize to one target are labeled, with two different fluorescent dyes—fluorescein and rhodamine. When the two probes hybridize to the target, the emission of fluorescein excites the emission of rhodamine. Each alone emits a certain color; together they emit a different one. "All this works within two minutes, in the same tube, with two [reagent] additions," said Taylor.

Jeffrey Chamberlain of Baylor College of Medicine (Houston, TX) described his success with multiplex PCR analysis in detecting deletions associated with Duchenne's muscular dystrophy (DMD). "Doing PCR on 0.5 µg of DNA, with 18 selected primers and 23 cycles, we can see whether there is a whole or partial deletion in the patient on a three-percent agarose gel," he said. "It takes only five hours to do." Chamberlain also set up PCR to study duplications in the locus, and carrier detection in females since DMD is an X-linked recessive allele. By fluorescently labeling PCR primers and putting the reaction products on an Applied Biosystems' (Foster City, CA) 370 automated sequencer, he was able to detect gene dosage by the size of the fluorescent peaks. Future multiplex analyses will allow detection of mutations in many loci.

Chamberlain also screened for point mutations by chemical mismatch cleavage. In this technique, known amounts of labeled PCR-amplified products from DMD patients were hybridized with control wild-type DNA. Osmium tetroxide and hydroxylamine detect single mismatches in the hybrids, and in the presence of piperidine cleave the hybrid. The products of the cleavage reaction are run on a gel. Competitive oligo PCR priming is another technique that allows detection of point mutations. In this case, amplification depends on the ability to prime at a polymorphic base. —Robin Eisner

THE SOURCE FOR CARBOHYDRATE CHEMISTRY

Unrivalled expertise and product lines to serve the life scientist

Pfanstiehl Laboratories is the world leader in isolation, synthesis and purification of carbohydrates—everything from simple sugars to blocked intermediates to polysaccharides. This broad product range provides the tools for synthesis of nucleosides, biologically active carbohydrates, and other biopharmaceuticals.

With a heritage of 70 years of specialization, Pfanstiehl scientists and skilled technicians are well-equipped to meet the constantly changing needs of today's dynamic life sciences. This expertise is available to you on a confidential disclosure basis.

Product capability from bench to pilot plant to bulk volume

Whatever your needs, we can meet your requirements on spec, on budget and on time. Meticulous attention is paid to analysis and product quality, with full documentation maintained in accordance with GMP Guidelines. Product purity and uniformity are assured, batch after batch. In addition, special ultra-pure products are available as "reference standards."

We stand ready to serve you as a reliable, confidential and experienced supplier for your specialty chemical needs.



PFANSTIEHL LABORATORIES, INC.

The Source for Carbohydrate Chemistry
1219 GLEN ROCK AVENUE
WAUKEGAN, IL 60085-0439

Tel.: 312/623-0370
(After Nov. 1989: Area Code 708)
FAX: 312/623-9173 TELEX: 25-3672 PFANLAB

Send me:

- New Capabilities Brochure
- Product Catalog
- Call us to discuss a project.

Area Code _____ / _____

NAME _____

TITLE _____

FIRM _____

ADDRESS _____

CITY _____

STATE _____ ZIP _____

50-R _____