New method may speed gene searches

To geneticists, the final frontier lies literally at our fingertips – and elsewhere in the body. Each of our cells holds a vast storehouse of genetic information in some 100,000 genes strung out along 46 chromosomes. Collectively this material forms the human genome.

Using DNA probes – snippets of genetic material that home in on specific segments of the genome – geneticists have explored the genes of people in families affected by various inherited illnesses, seeking the defect that causes their disease. These "linkage studies" have scored important successes, but they remain painstakingly slow to carry out.

Now, a new technique, genomic mismatch scanning (GMS), may provide geneticists with a more rapid and precise means of finding genes. The technique uses a series of enzymes to isolate identical stretches of DNA – the thread-like molecule that holds the genetic code – from different individuals. Knowing the regions of DNA shared by two related individuals – possibly inherited from a common ancestor – helps geneticists find the particular locations on chromosomes that carry disease genes.

"GMS makes it appear feasible to go after a lot of genetically complex traits that would be ridiculously expensive and laborious, and maybe impossible, to do by any other means," says Patrick O. Brown of Stanford University Medical Center. Such complex, multi-gene traits include susceptibility to schizophrenia.

Brown and his colleagues describe their use of the technique, which they developed, to study inheritance patterns in yeast in the May issue of NATURE GENETICS. The researchers are now working out the details of how to use GMS on the larger, more complex human genome.

GMS uses a series of enzymes, including several from the DNA-repair machinery of the bacterium *Escherichia coli*. Researchers break up the chromosomes of two individuals into thousands of fragments, unzip their two mirror-image strands of DNA, and then allow the separated strands to regroup, sometimes as a mixture of strands from two individuals. Then, enzymes eliminate all combinations but those that contain identical genetic information from two individuals. Scientists can use these surviving fragments to identify the DNA that two individuals inherited from a common ancestor.

In conventional linkage analysis, researchers use previously mapped reference points on chromosomes, called genetic markers, to narrow the search for a gene to a general region of a chromosome. These markers often pass in identical form from parent to child along with nearby genes. After years of scrutinizing the DNA of related individuals affected by a disease, researchers can find markers that point to the gene's approximate location.

According to Stanley F. Nelson, a postdoctoral fellow in Brown's laboratory, researchers using conventional linkage analysis may have to check scores of markers, one by one, to track down an inherited trait. "The biggest advantage GMS offers is the ability to scan an entire genome all at once," says Nelson.

But a formidable obstacle currently blocks widespread use of GMS on human DNA: The human genome contains a great deal of duplicate information. These repetitious DNA segments interfere with the GMS process, hindering its ability to create useful amounts of perfectly matched DNA fragments. The researchers believe they can solve these problems, however, and the results of preliminary experiments using GMS on human DNA are "encouraging," Brown says.

Dramatic proof of GMS' usefulness may come when scientists use it to track down genes responsible for an inherited disease, comments Jean-Marc Lalouel at the University of Utah in Salt Lake City. Lalouel and his colleagues are working to understand the genetic basis of high blood pressure (SN: 10/10/92, p.230).

"If the [GMS] method could be adapted to the human case, the impact could be very large indeed," says Stanford geneticist David Botstein. *– D. Pendick*

Exxon's *Valdez* studies ignite controversy

Alaska's Prince William Sound "has almost fully recovered from the 1989 *Exxon Valdez* oil spill," assert officials with the Houston-based Exxon Co. USA. That assessment, based on a spate of new papers by company-funded researchers, provoked an immediate flurry of heated charges and countercharges last week.

Exxon scientists say their data indicate that widespread oil contamination has plagued Prince William Sound for more than a century. They interpret these findings to suggest that area aquatic life can coexist with low levels of oil – and even recover from occasional heavy oiling.

Government claims of long-term *Exxon Valdez* damage usually can be traced to a "faulty interpretation" of data, Exxon argues in a statement it released April 26. As a result, the company says, government scientists have mistakenly assumed "that large numbers of biologic and sediment samples from Prince William Sound contained remnants of *Exxon Valdez* crude when, in fact, they did not."

Exxon issued its statement at the opening of a four-day environmental session at an American Society for Testing and Materials (ASTM) meeting in Atlanta. Researchers funded by Exxon presented 25 papers there on the *Valdez* spill.

However, some of those same papers lead chemist Jeffrey W. Short of the National Marine Fisheries Service in Juneau, Alaska, to suspect that there is at least some possibility that Exxon is ascribing to other sources a portion of the oil that actually came from the *Valdez* spill.

His concerns involve studies that attempted to identify the source of an oil from the chemical fingerprints of its polycyclic aromatic hydrocarbons (PAHs). These PAHs offer a relatively stable oil signature – one that persists even after a sample has weathered, or begun to turn tarry.

In one study, chemist David S. Page of Bowdoin College in Brunswick, Maine, and his co-workers assayed PAHs in more than 2,350 seafloor sediments collected in Prince William Sound and the adjacent Gulf of Alaska between 1989 and 1991.

Coauthor A. Edward Bence, a geochemist with Exxon in Houston, recalls how surprised he was to find a consistent background signature of crude oil – one quite different from the *Valdez* oil – going back at least 160 years throughout the supposedly pristine sediments in Prince William Sound. The age of the signature argued for some natural, continuing source of this petroleum.

Realizing that crude-oil seeps had been charted in several places along the eastern Gulf of Alaska, the researchers compared fingerprints of oil from the seeps to those of oil in Prince William Sound sediments. They matched.

Other Exxon studies offered an explanation of how the seeps' oil might have entered Prince William Sound. Clays from glaciers to the east readily combine with oil – especially weathered oil – to form flocculated emulsions (see story, p. 302). These buoyant floc particles would ride west on the Alaska coastal current (see diagram on facing page) until they hit the sound's slow waters and settled.

The Exxon survey of sediment fingerprints revealed large amounts of petroleum PAHs – concentrations sometimes approaching 500 to 1,000 parts per billion. In deep areas, most of the oil appears to have come from seeps. In shallower zones, diesel fuel was often present. And because this diesel oil, perhaps spilled during refueling, bore a signature quite similar to that of the *Exxon Valdez* oil, government chemists often mistook the two, Bence contends.

That's definitely possible and reflects "the bias I entered with," concedes Short, who led some of those analyses. If an oil fingerprint bore the distinctive PAH peaks representing phenanthrenes and dibenzothiophenes – characteristic of North Slope crude oil – "I assumed it was *Exxon Valdez* oil," he says. In fact, North Slope diesel oil contains the same two PAH peaks. Exxon differentiated between the

two oil types by looking for chrysenes: The diesel lacks these PAHs.

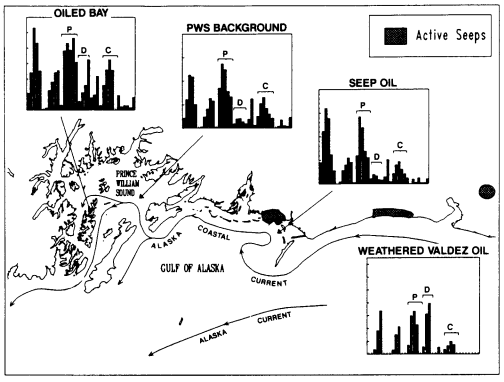
Short's own PAH fingerprinting supports Exxon's finding of background contamination with non-North Slope crude oil in deep Prince William Sound sediments – those in 40 to 100 meters of water. But his studies indicate that shallow, intertidal sediments generally remained pristine – totally free of petroleum residues – unless or until oiled by the *Exxon Valdez* spill.

And that's why at least one paper at the ASTM meeting bothered him. It attributed most of the PAHs in biological materials from one tidal area to a mix of seep oil and North Slope diesel. If one assumes such shallow areas contain a mix of diesel, seep, and *Valdez* oil, he says, then one can attribute much of any phenanthrenes and chrysenes present to seep oil, and much of the dibenzothiophene signature to diesel. This would allow you "to cover most of what you see with non-*Exxon-Valdez* oil – even if *Valdez* crude is the only [oil] present," he says. That prospect, he adds, "makes me suspicious."

Many government scientists lauded Exxon for the science reported at the ASTM meeting. Like Short, however, many also questioned how data from that research were being interpreted.

For instance, Exxon claims that "[oil] spill effects were not significant to the herring." But Evelyn D. Biggs in Alaska's Department of Fish and Game in Cordova says even Exxon's data don't support that claim. Her own histopathology studies show that "the tissues of fish in the oiled areas are more screwed up than tissues from fish in unoiled," she says.

Dennis Heinemann, a Camarillo, Calif.-based consulting seabird biologist, objects to the way Exxon's "careful," but limited, study on murres – a diving seabird – ignores conflicting findings from bigger, longer observations of those same birds (SN: 2/13/93, p.102). He compared Exxon's efforts to "looking at one tree" and then generalizing that conclusions drawn from it could "represent the whole forest." *–J. Raloff*



Adapted from Exxon/Page et al.

Shaded areas on map depict natural seeps that can contribute oil to Prince William Sound sediments. Chemical fingerprints illustrate how relative peaks of certain PAHs – phenanthrenes (P), dibenzothiophenes (D), and chrysenes (C) – differentiate Exxon Valdez *residues from seep oil.*

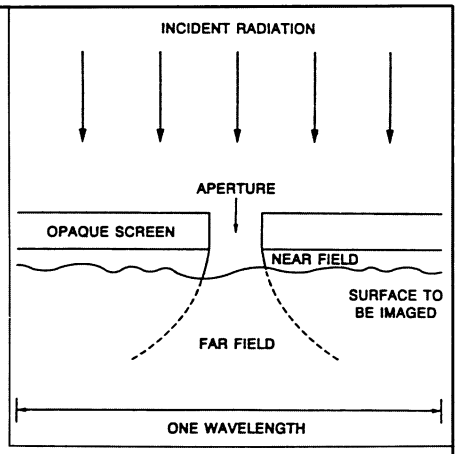
Close-up views of cells

Over the centuries, microscopists have developed many different techniques for staining biological material to highlight certain features of interest in tissue or within individual cells. The same techniques may now prove useful with a new type of optical microscope that produces sharp images of objects smaller than the wavelength of light used to illuminate the sample.

When light passes through a tiny opening, it tends to spread out, or diffract. This optical effect limits a conventional microscope's resolution. But by making the distance from the aperture to the surface being viewed much smaller than the wavelength of the illuminating light, researchers can evade the diffraction limit and generate high-resolution images of surfaces (see diagram).

Eric Betzig of AT&T Bell Laboratories in Murray Hill, N.J., and his collaborators send visible laser light through an aluminum-sheathed optical fiber tapered to a fine point 70 nanometers wide and positioned only 10 nanometers above the sample. Moving the glass fiber tip back and forth generates an image that reveals components as small as 15 nanometers across. In tests of their instrument, the researchers have obtained remarkably detailed images of the skeletal scaffolding inside a cell.

Betzig described his group's preliminary results at the Quantum Electronics and Laser Science Conference, held this week in Baltimore. □



Betzig

An illuminated aperture acts as a light source that can scan a surface to produce a high-resolution image.

Collapsing clusters lead to fullerenes

Chemists have quite successfully cooked up large quantities of fullerenes for three years now, but no one yet knows how these structures manage to emerge out of the hot carbon chaos. Why the commonly used arc-reactor-synthesis method works at all still mystifies researchers. How could atomized carbon spontaneously yield such highly ordered molecular cages?

New experimental evidence suggests that at high temperatures large carbon clusters form and then collapse into a more stable fullerene configuration.

"We've shown how carbon in a very high-energy environment reacts with itself and goes on to form fullerenes," says Michael T. Bowers of the University of California, Santa Barbara. "It's not what people – very reasonably – thought in the past."

The smallest observed fullerenes, containing 30 carbons, had appeared to come out of nowhere, Bowers says. He and his co-workers set out to discover how they form. Using a method called ion chromatography – which they developed to study carbon clusters – the team first determined what structures carbon atoms prefer to adopt.

Researchers had theorized that fullerenes assemble from sheets of pentagons and hexagons, but the group found no evidence of this. Instead, they observed that a few carbon atoms will link up linearly and that 10 carbons form monocyclic rings, 20 or more carbons form bicyclic rings, and 30 or more carbons form tricyclic rings.

In the May 6 NATURE, the California group describes how heating these large planar rings causes them to rearrange into the three-dimensional, spherical fullerenes. The rings melt down and a small carbon fragment evaporates as the atoms settle into their new arrangement.

Bowers speculates that in the searing carbon soup of an arc reactor, the 60-carbon buckyball forms preferentially because it is the most stable fullerene in the intermediate size range. Larger fullerenes may coalesce just outside the arc's hottest region, where negatively charged carbon clusters may lose electrons and grow further before melting into fullerenes.

Robert F. Curl of Rice University in Houston applauds the work for contributing to a fundamental understanding of fullerenes and for opening up theory to experimental testing. "Here's something that may bear very strongly on the formation of C_{60} and fullerenes in general," he says.

Such research may one day help chemists control the kind of fullerene they produce. Says Bowers, "Once you know the mechanism, you have a chance at tailoring molecules. Until then, people are just playing in the dark." *–K.F. Schmidt*