MOLECULAR BIOLOGY

A Faster Walk Along the Genome

If there's to be any hope of completing the Human Genome Project on schedule and within budget, geneticists agree they need more efficient ways to sequence the genome. But many experts doubt that the currently favored "shotgun" method—which relies on sequencing a random selection of overlapping DNA fragments, then piecing them together—will ever become much faster and cheaper. Larger gains could come, say some sequencers, from a new twist on a technique called primer walking, in which geneticists move steadily along a longer strand of DNA, sequencing contiguous stretches of it.

So far, the time and expense needed to make the primers—short DNA sequences that define the starting point for each sequencing run—have handicapped the technique. Now, however, several groups claim to have eased that step. If the technique lives up to its promise, says one of the researchers, Mathias Uhlén at the Royal Institute of Technology in Stockholm, it could lower the cost of sequencing by a factor of 10 or more and produce similar gains in speed.

The problem with the shotgun method is that it contains two bottlenecks. The first is the initial step of cloning the many different fragments of a DNA strand into sequencing vectors—typically circular strands of DNA from a phage virus. The fragments are sequenced by adding a short primer, usually consisting of 15 or more bases, that binds to a known sequence in the vector and serves as the starting point for producing a series of strands complementary to the unknown DNA, which can be analyzed by gel electrophoresis to read the sequence. Then comes the second bottleneck: figuring out where each fragment fitted on the original, continuous DNA.

Primer walking, in contrast, has only one cloning step: A single stretch of DNA is inserted in its entirety into a larger vector. And there's no need to piece together random fragments of DNA, either. Instead, after their first sequencing run, geneticists make a new primer that binds near the end of the stretch of DNA they've just sequenced—and repeat the process over and over until they've sequenced the whole of their unknown DNA.

Large-scale sequencers have turned their backs on primer walking in the past, however, because making a custom-designed primer for each sequencing run introduces lengthy delays. Now, four groups are testing libraries of ready-made modules, which join up into effective primers at the right spot on the template.

In 1990, Waclaw Szybalski of the University of Wisconsin proposed in a paper in *Gene* that the ideal modules might be six-base sequences called hexamers, which come in only 4096 possible unique forms. Molecular geneticist William Studier of the Brookhaven National Laboratory was the first to put that suggestion to the test. As he reported late last year in *Science* (11 December 1992, p. 1787), he and two colleagues coated a DNA template with a DNA binding protein, then added the three hexamers that, combined, would form the desired primer sequence. Studier found that the hexamers displaced the protein only where they could bind to the template side by side, mimicking an orthodox 18-base sequencing primer.

In the 1 May *Proceedings of the National Academy of Sciences*, Levy Ulanovsky and his colleagues at the Weizmann Institute in Rehovot, Israel, report a similar success—only these researchers managed to prime sequencing reactions using strings of three hexamers even without shielding the template with binding protein. Meanwhile, the two remaining groups, one led by Uhlén and another led by Szybalski, have added the extra step of covalently bonding their hexamers together after they've bound to the template.

All four groups are now working furiously toward the same goal: automation. That should make it possible, Szybalski says, for a group of fewer than 10 people to sequence the entire genome of the bacterium *E. coli* within a few weeks—a task that would take several years with today's technology.

Most big-time shotgun sequencers are much more cautious, however. "It's interesting," says John Sulston, head of the Sanger Center in Cambridge, England, home of one of the world's largest sequencing initiatives. "But it's yet to be proved on a large scale." He's particularly worried that the frequent repeated sequences found in the genomes of higher organisms will pose a major obstacle, causing the hexamers to bind at multiple positions along the same template.

The primer walking enthusiasts acknowledge the problems but remain confident that they can be solved. "This technology is at the stage those people were at 3 to 5 years ago," says Studier. Within a year or two, he's confident that primer walking will be a "bread-and-butter technique" for the world's large-scale sequencers.

–Peter Aldhous

RESEARCH IN JAPAN

Computer Firms Look to the Brain

In a cramped laboratory in the western suburbs of Tokyo, researcher Akio Kawana sends pulses of electricity across his specialized culture dishes and measures the faint electrical signals given off by nerve cells as they communicate with one another. The scene itself is not unusual. Scientists doing similar work can be found in many neuroscience labs at universities and research institutes around the world. But Kawana doesn't work in an academic laboratory. His employer is Nippon Telegraph and Telephone (NTT), the world's second largest telecommunications company (AT&T is first). Why would a former optical fiber engineer at an electronics giant like NTT be interested in nerve cell behavior? And here's a better question: At the very moment in scientific history when the premier U.S. corporate research labs are shutting down basic research efforts, why would NTT encourage work so seemingly unrelated to its business?

Neural learners. Brain researchers *(from left)* Fukunishi, Sekiguchi, and Tanaka.

And NTT is not the only Japanese company to which the question applies. Rather than relying just on academic research, several of Japan's corporate giants are betting that pure research today will guarantee leadership tomorrow in computers. Since present-day computers are bad at higher forms of information processing, such as recognizing spoken language, a person's face, or making logical inferences about what they are told—abilities that children have but which elude multi-million-dollar supercomputers—Japanese corporate research directors are looking to mimic the human brain's information processing capabilities. Enter Kawana—who hopes that what he learns from small networks of nerve cells will help him understand how information is