Genome Mapping Goal Now in Reach

James Watson has promised to complete a map of the human genome within 5 years; now it looks like it might be doable

SCIENTIFIC PROGRESS is often based on a hefty share of blind faith, and the genome project promises to be no exception. The oft-stated first goal of this new project is to complete a detailed map of the human genome, a guide to where the genes are on all 46 chromosomes. "I have stuck my reputation on getting it done in 5 years," said James Watson, associate director for human genome research at the National Institutes of Health, this spring. The catch is that no one has known how to meet this timetable with current technologies.

Now that may have changed with the advent of two new—and still quite preliminary—mapping techniques, one developed by Yale geneticist David Ward and his colleagues, the other by David Cox and Richard Myers of the University of California at San Francisco. "I'm optimistic," says Watson. "It means we can actually get the physical map done in 5 years." Eric Lander of the Whitehead Institute in Cambridge agrees: "It is no longer rash to say we can do it in 5 years."

The map is certainly the project's most tangible goal, as it promises near-term benefits in tracking down the genes that cause major diseases, such as cystic fibrosis and Huntington's disease. It is also attractive to congressmen. "The people giving the money would like it done in a reasonable time," said Watson, who noted that given the age of most congressmen, "they want DNA in a bottle to go after the Alzheimer's gene."

There are essentially two types of maps that may yield up the putative Alzheimer's gene and other long-sought disease genes. The first, the genetic linkage map, shows the arrangement of genes and markers along the chromosomes as calculated by the frequency with which they are inherited together. While it provides a powerful tool for narrowing the search for disease genes, it is not sufficient for actually plucking out the gene and analyzing it. That, eventually, should come from the second map.

Termed a physical map, this is an actual representation of the chromosomes, providing the physical distance between landmarks on the chromosome, ideally measured in nucleotide bases. Physical mapping involves lining up pieces of DNA—the "DNA in a bottle" Watson referred to—in the order in which they appear along the chromosome. The ultimate physical map is the complete sequence itself, the exact order of the 3 billion nucleotide bases that make up the human genome. Achieving the sequence is likely to take 15 years and cost $3 billion.

Of the two types of maps, the genetic map is much further along. The entire genome has been blanketed with numerous landmarks spaced, on average, 10 centimorgans apart. (A centimorgan is a measure of genetic distance—how often two markers are separated during meiosis—but it roughly translates into a physical distance of 1 million bases.) In some places, however, there are clusters of closely spaced markers; in others, there are huge gaps.

Efforts are under way to build a finer resolution, 5-centimorgan map. And Watson has said he wants a 1-centimorgan map, with markers spaced roughly 1 million bases apart, complete within 5 years—a goal that Maynard Olson, a geneticist at Washington University, calls "achievable but fairly horrendous."

That pales, however, when compared with the difficulty of completing a detailed physical map, which has never been attempted for anything as large as the human genome. Bits and pieces of the human genome have been mapped in detail, generally around known disease loci, and efforts are under way to complete physical maps for several human chromosomes. But no one has even considered tackling the entire human genome, at least not until recently.

Greg Beck

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Enter Ward and Cox and Myers with their new mapping strategies—one a physical mapping technique; the other, a genetic/physical hybrid. Cox and Myers are zapping chromosomes with x-rays and then determining the order of genes along the chromosomes by how often they are separated by these x-ray breakpoints. Ward and Peter Lichter, whom Ward credits with having done much of the work, are tagging DNA fragments with fluorescent dyes and then visualizing the order in which they hybridize along the chromosomes (see box).

Cox first described their new strategy about a year ago at a meeting at Cold Spring Harbor Laboratory (*Science*, 3 June, p. 1278) and provided new details at a February workshop at NIH. Ward announced his group's technique at the recent NIH meeting. Neither has been published, but already, labs are gearing up to try them out.

Ward and Lichter's technique is a refinement of the technique of in situ hybridization, in which a DNA probe is labeled with a radioactive tag and hybridized to a chromosome to see where it sticks; it will seek out and bind to its complementary sequence. Instead of labeling DNA probes with radioisotopes, Ward and Lichter tag them another way that can be detected with fluorescence and then hybridize them to a metaphase chromosome. "We are actually visualizing where genes are on a chromosome," says Ward. Through a light microscope the probes show up as bright yellow-green dots against the chromosome, which has been stained red.

"The advantage is speed," says Ward. "You can change the process of mapping a gene from months to overnight." In the past 6 months, with just three people working on it, Ward's lab has already used this approach to determine the order of about 100 probes and genes on chromosome 11.

This approach also offers "vastly superior resolution" over in situ hybridization, says Ward, who can now distinguish two probes as close as 1 million base pairs apart. With new equipment he expects to resolve probes a mere 30,000 bases apart. Two other groups are working on a similar mapping technique, one led by Evani Viegas-Pequigot of the Institut Curie in Paris, the other by Dorra Cherif of the Hospital of St. Louis in Paris.

At this stage, Ward's lab is still mapping one probe at a time, though the process is

amenable to doing six or eight at a pop, says Ward, with each one tagged with a different dye and emitting a different color. First, though, they need equipment capable of resolving that many colors. "If we got that equipment and had four or five people working on it, our lab could map 4000 or 5000 genes a year," asserts Ward.

Cox notes that Ward's technique, like his own, is still new and somewhat uncertain. "Ward has the resolution to hybridize two probes at the same time and see the distance between them—but can it be generalized? He has pretty pictures, but will it always work? Like our technique, it remains to be seen."

One reason for the excitement surrounding these two new strategies is that both promise to provide a way to complete what is called a contig map, a widely used physical mapping technique, far sooner than anticipated. Contig mapping involves fragmenting DNA into tiny pieces and then using computer techniques to search for overlaps, or shared sequences, to line up the fragments in correct order. This can provide a detailed map of a small region of the chromosome. The problem is that this approach yields groups of overlapping clones, known as contigs, with often huge gaps in between them. Nor does it necessarily tell you what chromosome the contigs are from.

If this approach is ever to yield a continuous, global map of the human genome, the trick will be to find a way to connect those islands and order the contigs along the chromosome. Until now, the only way to anchor the contig map has been to use the genetic map for orientation. Indeed, that has been one reason for the push for a linkage map of increasingly fine resolution.

Now, says Watson, such a fine-resolution genetic map may no longer be necessary. "We may want a 1-centimorgan map in its own right to search for disease genes," says Watson, "but we won't need it to correlate the physical map."

"I expect this will enable us to order contigs more easily," agrees Lander, who cautions that "it is by no means settled. The other approaches are new, the artifacts have not been worked out, we are not sure how reliable they are. The genetic map is still a crucial way to order the physical map."

Olson, however, is not convinced that pasting together tiny pieces of DNA is the way to create a global map of the human genome. "Contig building is the bottom-up approach. You start with highly fragmented DNA and build a more and more continuous map. I think that will play a minor role at best in mapping the human genome. It hasn't achieved anything like even moderate-range continuity and I doubt it ever will."

To Olson, the beauty of both Cox and Ward's techniques is that they may make it possible to get a continuous, albeit relatively low-resolution, map of the entire genome, and from there to work toward greater resolution—a "top-down" approach.

And that is what Ward is proposing to do. His grand scheme, as he calls it, is to do "saturation hybridization"—to map 5000 or 7000 probes to create landmarks spaced, on average, 1 million base pairs apart across the genome. "We view it as a way to get defined and localized anchor points across the genome as quickly as possible." Ward would then make those clones available for finer resolution genetic or physical mapping. Cox and Myers' strategy, too, can be used to create a continuous map with varying levels of resolution, depending on the x-ray dose they use.

"I'm very enthusiastic about all these techniques," says Olson. "My view is that the physical map will be so hard that we need all the methods we can get—in situ hybridization, linkage mapping, radiation mapping, and two or three more as well. No one of these is powerful enough to guess it will have a high likelihood of global success."

Adds Lander: "The nice thing is that 5 years ago there was some speculation about when, if at all, we would have a 5- or 10-centimorgan genetic linkage map. It fell together. And now we are beginning to reach that stage with the physical map. Rudimentary maps of human chromosomes may now be possible." ■ **LESLIE ROBERTS**

Mapping by Color and X-rays

David Ward's new mapping technique is, in essence, a souped-up version of conventional in situ hybridization. The standard way to map a gene is to put a radioactive tag on a probe and hybridize it to a chromosome. To see where the probe goes, the investigator must then overlay the chromosome with a photographic emulsion and expose the slide for weeks or months. Says Ward: "Using our method, you can do the experiment overnight."

Rather than tag their DNA probes with radioisotopes, Ward and colleague Peter Lichter label them with a "reporter" molecule, a small molecule to which there are active binding proteins. The process works this way. They take a small piece of DNA and label it with, say, the vitamin biotin. That probe is then hybridized to a metaphase chromosome spread, where it will seek out its complementary sequence. Meanwhile, the investigators tag the binding protein—in this case, avidin—with a fluorescent dye. To find the probe's location, the investigators incubate the chromosome spread with the fluorescently tagged avidin, which then binds with biotin. "The next morning you see where the gene is," says Ward, which shows up as a bright yellow-green spot.

In addition to speed and resolution, this approach has another advantage over conventional in situ hybridization, says Ward: it gets around the problem of repetitive sequences. Much of the drudgery of in situ hybridization comes from the need to remove the repetitive sequences, which would confound the signal by hybridizing in many places. Ward and Lichter have come up with a way to repress the signal from repetitive sequences without actually removing them. They take a clone, mix it up with human DNA, and "preanneal" it, which involves separating the two DNA strands and allowing them to reassociate. Because of their abundance, the repetitive sequences will find their partners and form a double strand much faster than the unique sequences. And once converted to doubled-stranded sequences, they will no longer hybridize.

David Cox and Richard Myers' technique, which they call radiation hybrid mapping, borrows from both genetic and physical mapping strategies. Instead of looking at how often two markers are separated during meiosis, as is done in genetic linkage mapping, they look at how often they are separated when the chromosome is zapped with x-rays and fragmented. This strategy is based on an idea proposed some 10 years ago by Henry Harris and Steve Goss that was virtually ignored.

Depending on the x-ray dose used, this approach can provide 20 times higher resolution than conventional genetic mapping, says Cox, as resolution is determined simply by how often the chromosome is broken. If a chromosome is zapped with 1000 rads, for example, breakpoints will appear every 50,000 bases, as opposed to the 1-million-base resolution sought with conventional linkage mapping.

Cox's description of this technique a year ago at Cold Spring Harbor engendered considerable excitement, even though he provided no evidence that the linear order this map predicts is in fact correct. "Now we have done the mathematics," says Cox, who gets odds of over 1000 to 1 that the order is correct. ■ **L.R.**