

Genome

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This Time it's Personal

The Promise and Peril of Personal Genomics

Whether or not personal genomics turns out to be hype, there's no question that 2007 was the year of the personal genome.

The DNA sequence of molecular bad boy Craig Venter was decoded last spring, published last fall, and is now regarded as the gold standard — the first "complete-ish" genome from a single individual to be sequenced at a high level of quality. In May, Nobel laureate Jim Watson's complete genome was deposited into GenBank. Another personal genome — this one from an anonymous Asian — was sequenced in October 2007. Another anonymous Chinese person and a Yoruba have since had their genomes added to the mix.

In 2006, Harvard geneticist George Church began recruiting a small group of subjects to undergo partial genome sequencing without the usual guarantees of privacy and confidentiality. His ultimate goal is 100,000 partial genomes, while Venter plans to sequence 10,000 complete ones in the next decade, starting with 30 or 40 in 2008. Meanwhile, the Archon X Prize for Genomics, a \$10 million award, has been promised to the first group to sequence 100 human genomes in ten days (with celebrity genomes to follow). Finally, at press time no less than six companies were offering (or about to offer) personal genomic services. Hard to believe that less than a decade ago, we were all atwitter at the thought of the first reference human genome, the product of the Human Genome Project that ushered in this new era.

Why this convergence now? Is it for real? And what should we expect?



Moore's Law on Steroids?

One of the obvious drivers of the rise in personal genome sequencing and genotyping has been the precipitous decline in costs. The price tag of the original Human Genome Project was \$2.7 billion for our 3.2 billion base pairs. By 2003, that cost had fallen to about \$150 million. Craig Venter estimates that, using the old-school sequencing technology of the late 1990s and early 2000s, his genome cost about \$70 million. But Jim Watson's genome, sequenced using next-generation technology, is thought to have cost less than \$2 million.

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"Swabbing" the Blue Devil for DNA.

Message from the Director

I know less about my genome than you might expect. From my own undergraduate senior honor's thesis, I know I have 46 chromosomes, including an X and a Y. From my father, I know (or I think I know...) that we can trace the family genome to the late 1500's in southern England. My eyes are blue and my blood type A-positive. And somewhere in my 3 billion or so units of DNA, I think, lurks a nasty bit of code that gave me colon cancer eight years ago, as it did to my father before me and his father before him. That's all I know, but all that's about to change.

Of all the promises about what's to come from our ever-increasing familiarity with the human genome and its global variation, surely the most eagerly awaited is personalized medicine. And yet, for all the breathless pronouncements of what might be in store, most in the medical community have to admit that we are far from using information on personal genomes in this way. Not surprising, perhaps, to those who have followed other

technology-driven changes in healthcare; the process is slow and measured by design as technology and innovation at the breaking edge of the wave are held back – even dragged back – by the medical community, regulatory agencies and the insurance industry.

What *is* surprising to many, though, is that the push to bring our genomes into clinical practice is coming as much or more from consumers as from professionals. Several companies are already offering genome-wide profiles direct to the public, some accompanied by risk estimates for various diseases. The investment community and media have responded rather positively to these offerings, largely ignoring loud "harrumphs" from experts who say it's too soon. It is clear that some among us, whatever their motivation, are ready to test their genomes.

What are they hoping to discover? Are they convinced that their genomes can foretell the future? Or do they just think it's kind of neat and

they want to be among the first in line? Will the information empower or unsettle them? Will they share it? With whom? Their spouse? Children? Neighbors? Doctors? Insurance companies?

We hope to find out. With the help of the Duke Blue Devil, we have just unveiled the Duke Personal Variome Project, designed to test a million "SNPs" in the genome (positions that show lots of individual variation) in Duke and Durham volunteers. The early stages will help us figure out how best to share this information with people, how to convey clear-cut results versus uncertain ones, and how to gauge individuals' responses to "seeing" their genomes for the first time. Some may want to parse every SNP and what it may or may not say about physical or personality traits, their medical past or future, or their family ancestry; some may choose to hide certain information, from themselves or from others. Some may change their minds altogether—what might have seemed like a nifty idea when they had their DNA collected may seem much less appealing with the envelope in hand. Pandora's box? Or a genie in the genome?

To be sure, the Duke Personal Variome Project is as much a social experiment as a scientific one, and we're fortunate to have a team of investigators who are experts both in the science and in its social and policy implications. The concept of Duke's IGSP came at the time of the original Human Genome Project, when the prospect of a reference genome sequence promised (or threatened) to change everything. But now – just a short few years later – it's real and it's getting personal.

But how can I ask others about their genomes or even pretend to comment on what it really means to know one, if I don't know mine? A fair point, and one I've thought about for years. So, in parallel with the Variome Project, I'm testing my own. What will my million SNPs tell me? Who will I tell? Will it be an epiphany or just another day in the lab?

I don't know, but all that's about to change. ▶

Huntington F. Willard, Director

Personal Genomics (continued)

And today? Kevin Shianna, director of the IGSP's genotyping core facility, says that with the new technology on the market (see story on page 7), the price will only get lower. Recently, next-generation sequencer developers Helicos BioSciences and Applied Biosystems said they could sequence complete human genomes at deep coverage for \$72,000 and \$60,000, respectively.

Harvard's George Church and others have compared this dizzying pace to Moore's law, which states that computing power doubles every two years. In a recent review, however, Clyde Hutchison (a sequencing pioneer and Venter colleague) observed that the amount of DNA and RNA sequence in public databases has actually doubled every sixteen months, easily outpacing Moore's law.

Genes 4 Sale

The steep drop in sequencing and genotyping costs has made it possible for private personal genomics companies to hang out their shingles. Late last year, deCODE Genetics, an Icelandic genomic discovery company, launched deCODEme™, a \$985-service designed to enable individuals to get a detailed look at their own genomes using hundreds of thousands of genetic markers known as single-nucleotide polymorphisms (SNPs).

Three days later, California-based 23andMe (named after the 23 human chromosome pairs) lifted the veil on its closely guarded business plan. For \$1000, the company lets individuals search and explore their genomes; learn how current research relates directly to traits identified in their genome; compare their genomes to family and friends who are also 23andMe customers; discover their genetic roots; and give them the option to actively participate in new research.

Not far from 23andMe in the Bay Area is Navigenics, expected to launch just as *GenomeLIFE* went to press. Its initial concentration will be on SNPs tied to select medical conditions. While it's content to use markers for now, Navigenics expects to eventually use whole-genome sequencing to analyze its customers' genetic endowments. 23andMe recently revealed that it had a number of individuals queued up and ready to spend \$250,000 to get their entire genomes sequenced. Last year, a source at 454 Life Sciences told *GenomeLIFE* much the same thing — people were prepared to pay 454 to decode their entire three billion letters of DNA. Meanwhile, Massachusetts-based Knome is offering standard whole-genome sequencing and comprehensive analysis services to individuals for a "mere" \$350,000.

A SNP: What is it Good for?

If the business community is gung ho about these services, the genome sciences, clinical and policy communities appear to be decidedly less so. A recent editorial in the *New England Journal of Medicine* pooh-poohed commercial personal genomics. The big question, says Geoff Ginsburg, director of the IGSP's Center for Genomic Medicine, is what one does with the information.

"If a patient brought me his genotype data, I would do my best to under-



George Church

stand the context and integrate it into his care," Ginsburg said. "But it still would not necessarily address the other issues: do we have enough information to actually make use of these tests? Have the associations with these markers been validated? Have they been replicated in multiple groups? If we take action because of them, will it change anything?"

For Ginsburg's colleague, David Goldstein, director of the IGSP's Center for Population Genomics & Pharmacogenetics, the last point is key. "We remain in a situation where we have very little that is clinically actionable."

Sharon Terry, director of the advocacy group Genetic Alliance, concurs; she thinks that marketing personal genomics to consumers at this point is simply premature. "I was asked to be part of this first wave of public sequences and I turned it down," she writes via email. "When having one's genome sequenced produces meaningful information based on real associations, then I would easily do it."

But without that certainty, says Assistant Research Professor and genome policy scholar Susanne Haga, the cons outweigh the pros for some people, including her. "It's probably not harmful, but it's not where I would feel comfortable," she says. "That might seem hypocritical coming from a geneticist, but it's an extremely personal decision. I'm in that group of people that would rather not know."

Are there exceptions? Goldstein mentions genetic testing for hypersensitivity to the HIV medication abacavir. HIV-infected patients carrying a certain version of the HLA-B gene can be extremely sensitive to the medication; if such patients are given abacavir and then subsequently re-challenged with it, they can die. "That shows there are genetic tests that can be clinically important," says Goldstein.

No Peeking?

Privacy is another hot-button issue for personal genomics. The National Institutes of Health has taken pains to reassure subjects participating in NIH-sponsored genome-wide association studies that their data will remain anonymous. So too have each of the personal genomics companies.

Property Rights...and Wrongs

The Center for Public Genomics Explores Intellectual Property

Professor XX has an idea for a new genome technology that she believes is a better mouse-trap and sure to make genomic research easier for thousands of investigators, grad students and postdocs toiling away at their benches. She would like to see it get into as many hands as possible.

What should she do with it? Run out and patent it? Publish it on the internet? Neither? Both? It is these sorts of questions the Duke Center for Public Genomics set out to address when it was established in 2004 within the IGSP's Center for Genome Ethics, Law & Policy (GELP) with five years of funding from the National Human Genome Research Institute.

History Lessons

Research Professor of Public Policy Studies Bob Cook-Deegan, who directs both GELP and the Center for Public Genomics, believes that the answers will come from taking a look back.

"Our approach is to look at technologies that everybody agrees are really important in carrying out genomics — DNA sequencing, microarrays, the seminal bioinformatics techniques everyone uses, and so on — and to examine how those innovations happened. We want to look at important technologies and build back from them to figure out: Did intellectual property help or hurt? What exactly was its role?"

When he gives talks, Cook-Deegan shows a slide of more than a dozen such technologies from sequencing to cloning to the polymerase chain reaction. Some remain unpatented, some have been patented by academic institutions and licensed either exclusively or nonexclusively, while others have been patented and tightly held by a single firm. "The question then becomes: what is the formula? It seems to me it's kind of like ecosystems biology in the sense that there isn't going to be one general law."

Beaded Lightning

Under Cook-Deegan's tutelage, Deirdre Parsons, a recent graduate student, traced the history of Illumina, Inc. to learn about the development of its powerful and proprietary BeadArray technology. In a single experiment, the BeadArray can be used to type hundreds of thousands of genomic variants called single-nucleotide polymorphisms (SNPs).

It originated with a Tufts University chemist named David Walt whose specialty was the use of fiber optics to detect different chemicals. He developed a novel way to create a random array of sensors and kept his idea a secret until he held the patent. (Earlier commercialization efforts had not been as successful as Walt had hoped). Once the patent was filed, he was approached by a venture capitalist named John Stuelpnagel, who identified the genotyping market as the one with the least competition.

The two men co-founded Illumina in 1998, and BeadArray technology has become one of two blockbuster genotyping platforms. Illumina has since expanded into other cutting-edge genomic technologies, most notably DNA sequencing via its 2007 purchase of Solexa (see story on next-generation sequencing on page 6).

Parsons believes much of the BeadArray's success can be attributed directly to Walt's savvy approach to intellectual property. "David Walt used his prior mistakes with intellectual property disclosure to decide how and when to disclose his invention," Parsons says. "His sophisticated decision-making process lies at the heart of the success of Illumina."

Cook-Deegan cites the Illumina story as a textbook example of the Bayh-Dole Act in action. Bayh-Dole was enacted by Congress in 1980 and, for the first time, gave US universities, small businesses and non-profits control of the intellectual property attached to inventions resulting from federally funded research.

"The Illumina BeadArray is your classic Bayh-Dole invention exclusively licensed to a startup company that then starts succeeding, gobbling up other technologies by buying other companies, and producing a product that everybody's using now," Cook-Deegan says.

Different Shades of Green

Professor of Law and IGSP member Arti Rai is exploring ways in which genome technologies are being distributed outside of the Bayh-Dole framework. At the top of her list is synthetic biology, the goal of which is to design and fabricate biological systems for engineering applications.

Synthetic bio has begun to attract serious money. Rai, for example, has been following the \$500-million investment made by BP (formerly British Petroleum) in green technology and synthetic biology under development at the University of California, Berkeley. The idea is to genetically customize microorganisms to make ethanol, biobutanol and/or other fuels from plant sources. In contrast to typical Bayh-Dole scenarios, observes Rai, BP is making a substantial corporate investment upfront and will likely exert strong control over the fruits of their investment.

"BP's economically rational self interest is not always going to coincide perfectly with the public interest," Rai says. "That's what we're concerned about — it's going to be a very interesting area to watch."

Another Brick in the Wall

Rai and James Boyle, William Neal Reynolds Professor of Law, are also tracking the progress of another synthetic bio initiative, BioBricks™, which are meant to be 'standard' DNA parts. Each BioBrick™ part performs a DNA- or RNA-encoded molecular biological function, such as switching gene expression on or off. Scientists can simply log onto the Registry of Standard Biological Parts

at MIT (parts.mit.edu) to learn about BioBricks™ in the current catalog.

Rai points out that rather than try to patent BioBricks™, the BioBricks Foundation (a not-for-profit organization founded by engineers and scientists from MIT, Harvard, and the University of California, San Francisco) is pursuing a trademark strategy. “They want to set up a standard to make sure people don’t get confused as to which BioBricks are the actual real ones. Like the Good Housekeeping Seal of Approval, trademarks prevent consumer confusion and, in this case, scientist confusion.”

Yea though I Walk through the Valley of Death...

Rai has come away with a profound sense of the limits of intellectual property in genomics. In talking to legislators and company representatives, she found that genomic startups are having an increasingly difficult time attracting early-stage funding from venture capitalists. That funding, notes Rai, is what companies need to traverse the so-called “valley of death,” the rocky path from invention to marketed product. During the biotech boom of a few years ago, a patent was enough to impress VCs. Not anymore.

“Everyone seems to have a genomic drug target and patents on it. It may be that the information underlying those patents is useless,” Rai says. “And, as [Frederick C. Joerg Professor of Business Administration] Wes Cohen has shown, it may be that if it does turn out to be useful, a pharma company will just infringe anyway. So these days VCs seem to be demanding Phase I clinical trial data. That’s a real problem because it becomes a chicken and egg issue: if you don’t get VC funding then you’re probably not going to ever be able to get to Phase I.”

One solution is for more government funding to help push young companies across the valley of death. Another, which Rai is working on with Bunyan S. Womble Professor of Law and Center for Public Genomics co-investigator Jerome Reichman, is a system of publicly funded public-private partnerships that would rely on more than intellectual property.

One Size Won’t Fit All

Cook-Deegan says that the Illumina story and Rai’s recent insights show that patents on genome technologies aren’t useless, nor a panacea. At the end of the day, the Center for Public Genomics’ job is to ferret out when patents do work well, and

identify alternative approaches to intellectual property that might be useful going forward. “Our mission is to think through the process without taking sides in the ideological debates about whether every patent is perfect or all patents are evil,” Cook-Deegan says. “We are trying to take a dispassionate view of how we as a society are going to be best off in the long run.”

For Professor XX, this may mean filing multiple patents and demanding that they be enforced aggressively. Or it may mean filing no patent whatsoever, letting her invention make its way in an unfettered, open-source manner. Or perhaps a little of both.

“Scientists like David Walt and others are very into open science, but at the same time they are very comfortable with commercial relationships. They have some intuitive sense about which kind of information should be handled in which way. How are they making those judgments? Those heuristics are what we want embedded in our system for dealing with genomic technologies.” ▶

Personal Genomics (continued)

At the same time, both the NIH and companies like 23andMe have stressed the importance of making their anonymized genomic datasets widely available to the research community. In a November 2007 conference call, 23andMe executives emphasized the ways in which their services will enable research. But is it reasonable to expect genomic data to be widely shared among researchers and invisible to everyone else except the subject himself?

“It’s a bit of a conundrum, isn’t it?” says Haga. “I think it can be kept private, but like any other security system, there will be occurrences where people hack into the system and figure out that Joe Citizen participated in a heart disease trial, here’s his DNA sequence and, lo and behold, he is at increased risk for heart disease.”

Harvard’s Church, who co-founded Knome and advises 23andMe, believes the risk for such security breaches in genomic studies has been downplayed. He notes that a social security number has only eight digits and a credit card 16, while a DNA sequence has three billion. For that reason, the consent form for

his Personal Genome Project (PGP) does not guarantee privacy or confidentiality. “We’re pretty good at computational security and we will try to keep the data private, but we’re not promising that,” he says. “It could become public.”

Initially, at least, it almost certainly will. The partial genome sequences of the PGP’s first ten subjects, including Church himself and the IGSP’s Misha Angrist, are likely to be posted on the internet in their entirety, just as Jim Watson’s and Craig Venter’s sequences are now available online. The PGP will also disclose subjects’ phenotypic information in the hopes of making the genomic data more useful.

Bob Cook-Deegan, director of the IGSP’s Center for Genome Ethics, Law & Policy, says these extreme views of privacy are to be expected. “Some people are completely comfortable with the world knowing everything about them except, say, their sexual practices and their income. Other people are going to be the reverse. I think we’re going to find out how intimate this information really is.” ▶

Talkin' 'bout My Generation

Next-Generation Sequencing Expands at Duke

Your version of the human genome has roughly three billion base pairs of DNA, plus or minus a few million. In 1990, the cost of knowing the identity of a single one of those bases was on the order of \$10. For that reason, decoding an entire human genome back then still seemed like a fantasy to many.

Today, reading that same base might set you back just a couple thousandths of a penny. Want a complete and accurate human genome sequence? Prep your samples and come back in a few weeks.

Much of the increase in speed and drop in price can be attributed to new methods of sequencing that have come to market in the past three years. Two of those methods are now available to genome scientists on campus.

"I'm very excited," says a slightly frazzled Greg Wray, director of the IGSP's Center for Evolutionary Genomics and the person overseeing integration of the new technologies into the institute's primary sequencing facility in the BioSci building. "But it's taken over my life."

Hedging Our Bets

Wray says there wasn't a particular application, set of scientific questions or group of organisms that motivated the IGSP to purchase the core facility's two new sequencers, the 454 Life Sciences Genome Sequencer FLX™, which is now "live," and the Illumina Genome Analyzer, soon to follow.

"It was more a sense that if you stand still you get run over," Wray said. "People were coming to me and saying, 'I'm tired of sending my samples out for sequencing, paying a lot of money, having no control over what happens to them, and then having to wait six months. Why don't we just get some of these machines ourselves?'"

As for the decision to buy two distinct technologies, Wray thinks it was a good way to hedge Duke's bets. "There's no question the two have different sweet spots." He notes that the 454 machine offers longer continuous sequencing reads and is therefore better for assembling genomes from scratch. It also requires just eight hours per 100-megabase run, whereas the Illumina machine takes three or four days.

Everything is Illuminated

On the other hand, Wray notes, the Illumina machine can crank out 1.4 billion bases per run and is likely to be better for resequencing known genomes like the human. It's also better for gene expression profiling.

"We all know that genes that are rarely expressed are difficult to detect using microarrays," Wray says. "But with sequencing on the Illumina platform, a single read will tell you whether it's there or not. Some people are predicting this will simply replace microarrays."



Kevin Shianna in the IGSP Genotyping Core Facility

One of those people is Kevin Shianna, director of the IGSP's genotyping facility, who recently purchased an Illumina machine on behalf of the Center for HIV-AIDS Vaccine Immunology and the IGSP (see story on page 7). "Digital expression is where it's at," he says. "As the cost comes down and we can run more samples at once, people will likely turn to the Illumina machine over microarrays because it has the potential to capture every transcript, even rare ones."

But if such a takeover is to occur, it probably won't happen within the next few years, said Director of the Duke Microarray Core facility Holly Dressman, citing the cost and limited availability of the technologies and other kinks still left unresolved.

"The tremendous amount of archival gene expression data now available is very valuable, particularly for translational genomics," she says. "Those who generate data with a new technology, such as Illumina, will lose the benefits of this rich source of existing data."

Start to Finish

Both Wray and Shianna emphasize that these are not your father's DNA sequencers: they require extensive sample preparation. Illumina users have to shear genomic DNA into small fragments, repair the ends of those fragments, and attach special adaptors for sequencing. The 454 system relies on fixing fractionated and adaptor-ligated DNA fragments to small beads in a water-in-oil emulsion. The DNA fixed to these beads is then amplified.

Because of these requirements, users will be expected to do more of the work upfront, which means constructing their own DNA libraries with adaptors

in place. “To be a successful core facility,” says Shianna, “we can’t try to take on every little bit. We can offer advice on how to do things, but we can’t actually do them for people.”

Wray echoes that sentiment. “My initial simplistic model is that we’ll have a series of documents and technical bulletins online. People will have to do more self-education. The applications are just too varied and the technologies too new for us to do complete handholding through the whole process.”

Just as sample prep can be overwhelming, the back end of the next-gen sequencing process is not trivial, either. The image files that are generated by the machines are terabyte-large – think of a hundred laptops’ worth of data with every run. Wray likens it to drinking from a fire hose.

“Fortunately,” he says, “the IGSP’s IT team has been incredibly helpful and professional. Jonathan Davis set up 28 terabytes of storage for us and a small cluster of processors to chew the data.”

IGSP Director Hunt Willard picks up the theme: “Riding a breaking wave is inherently messier and costlier than coasting along later on calmer seas. Investigators, the IGSP and Duke as a whole will all have to have skin in the game, whether it’s the up-front costs of the technology or the downstream costs of data storage and analysis. We may get lots of spray in our face, but I’m betting it’ll be well worth it. It wouldn’t mean much for us to want to be ‘almost’ cutting edge. That’s just not Duke.”

The Soul of the New Machines

However daunting the sample prep and data analysis, at least initially, Wray makes it clear that anyone with a good idea for a sequencing project should not be intimidated by the new instruments.

“We really want people to experiment with them — that’s the whole point,” he says. “We want to lower the price so folks are willing to come in and try them out, do different things, be creative, get something new going. Every single person in my own lab has something they want to do with these instruments. They have people thinking about their science in a new way. That’s what we want to achieve.”

Yes, but how do they perform? At a recent Genomes@4, Associate Professor Fred Dietrich presented data on complete resequencing of a fungal genome using the Illumina platform as carried out by a European commercial sequencing firm. He found the error rate to be acceptably low, the genomic coverage deep, and the alignments accurate. His conclusion: for resequencing microbial genomes at least, the Illumina machine really works.

IGSP Investigator and Assistant Professor Jun Zhu is a former postdoc in George Church’s lab and no stranger to various sequencing technologies. When asked what advice he would give to those contemplating next-gen sequencing, he is circumspect: “At the end of the day, you still need a good question to ask.” ▶

Seven Billion Genotypes...and Counting

The IGSP Genotyping Core Facility is already churning out data – lots of data. Kevin Shianna estimates they’ve captured seven billion genotypes thus far, and counting.

“We can now run 200 million every week,” he said. “That means another billion every month.”

And, he says, those numbers will climb yet again when Illumina comes out with a new chip capable of running more samples at once. “At full capacity, running five or six days a week, we will be able to run 240 chips—up to 480 million genotypes—per week,” Shianna said.

So what exactly are all the genomic data the facility has been generating in what has become one of the busiest academic genotyping operations in the country?

Shianna says he and David Goldstein, director of the Center for Population Genomics & Pharmacogenetics, have worked on several projects in areas including schizophrenia, chronic obstructive pulmonary disease and epilepsy. Then there are projects that various other Duke investigators bring to the core facility: Associate Professor of Biology and IGSP member Mohamed Noor’s fine-scale mapping of recombination rate in *Drosophila* is one example.

But most of Shianna’s effort is focused on parsing out the parts of the genome that can confer protection against HIV. That endeavor already bore fruit in a report led by Goldstein that appeared in *Science* last summer, which identified two gene variants in the DNA of 486 HIV-infected people that appeared to help control the virus and another variant related to its progression.

In search of more clues, Shianna said he and Goldstein are now set to start another genotyping study exploring the genomes of people in Malawi who have almost certainly been exposed to HIV repeatedly without getting infected. And in a second study, they will genotype people with severe hemophilia A who received concentrated clotting factor VIII before screening of donated blood was routine.

“A single dose of factor VIII is typically derived from the blood of thousands of people, and about 80 percent of hemophiliacs who received it before 1984 are infected [with HIV],” Shianna said. “We’re interested in that other 20 percent.”

For further information on the IGSP Genotyping Core Facility, see www.genome.duke.edu/cores/genotyping. ▶