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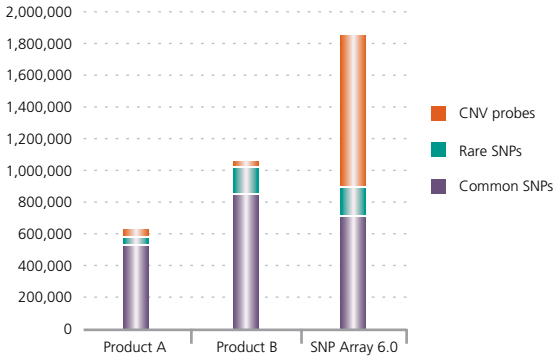

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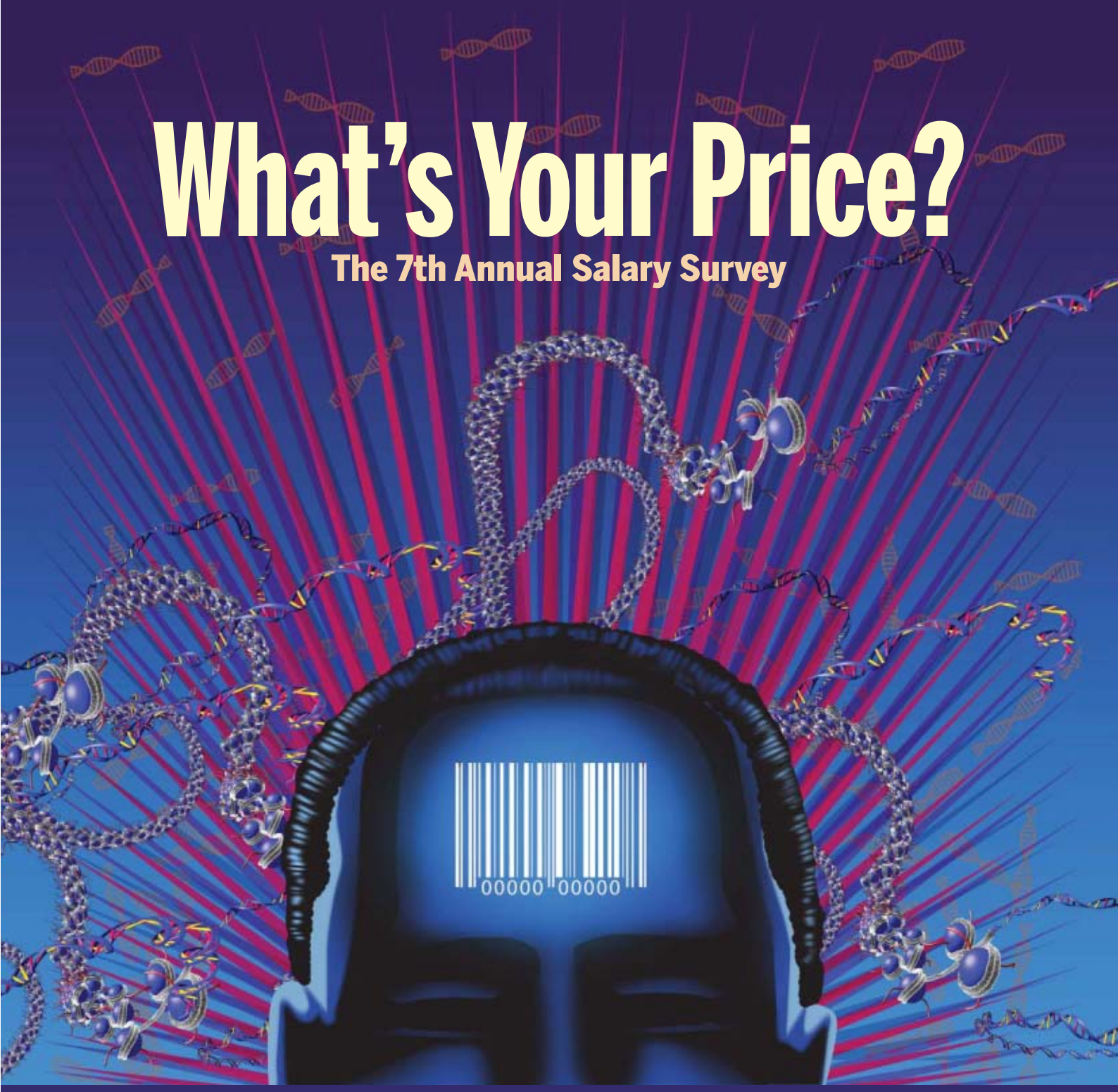

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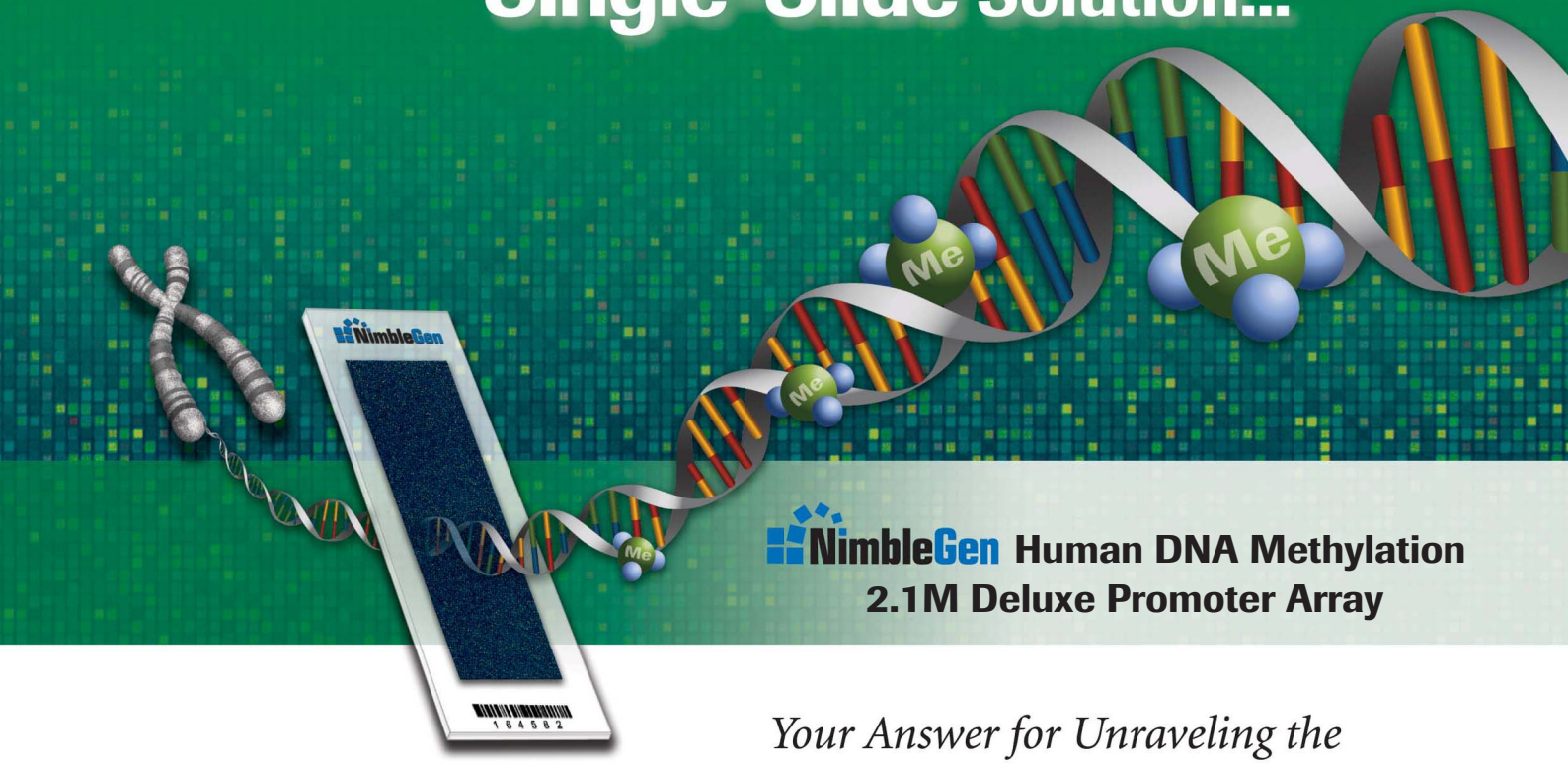

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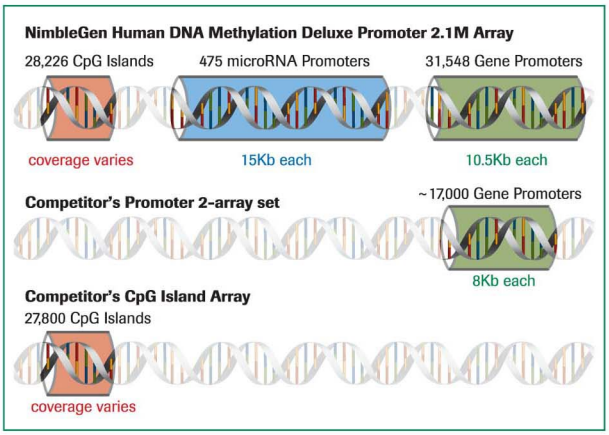

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Here's to You

As more biotech companies have battened down the hatches to survive this recession, it hasn't been surprising to see rounds of layoffs in industry — particularly as mergers and acquisitions have become more common in the field. What has come as a surprise to many scientists are the rumors of coming layoffs in academia. Even institutions that seem quite stable have watched their endowments dwindle and are going into cost savings mode.

It was in this gloomy environment that we sat down to plan our seventh annual salary survey. It's the only resource of its kind — targeted at scientists in the systems biology community — and we wanted to acknowledge that the landscape's a little different this year. So for the first time, we split out the survey with questions for people who have jobs and people who don't. (We all breathed a sigh of relief on seeing that just 3 percent of respondents reported being unemployed. That number is undoubtedly a bit lower than it should be, as some unemployed people won't bother taking a salary survey, but it's a far cry from the general unemployment stats we're routinely seeing in mainstream media.)

Many thanks to the 1,468 of you who responded to the survey this year, giving us all sorts of great data to showcase in this issue. Thanks also to those who took the time to send us your career questions, which we ran past experts in the field to get insight into how to handle salary negotiations, factors to consider when working abroad, why there's disparity in pay between men and women, and much more. You can check that out on p. 42.

Also in this issue, don't miss our feature articles on innovations in qPCR and new studies of genome-wide methylation enabled by microarray and sequencing advances. And for those of you who have been following the debate over the relevance of GWAS data, we've got a Q&A in this issue with Andrew Singleton at NIH, as well as a tech guide on best practices for running and analyzing association studies.

Meredith Salisbury

Meredith W. Salisbury, Editor

What do you think of Genome Technology*? Let me know how we're doing by e-mailing me at msalisbury@genomeweb.com or by calling me at +1.212.651.5635*

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COMMENTS FROM THE DAILY SCAN

In response to a post on the recent UK government decision to keep DNA samples from innocent people for up to 12 years, with which DNA fingerprinting pioneer Sir Alec Jeffreys disagrees, a reader wrote:

"Sir Alec is absolutely right. This database of DNA of innocent people is a disgrace, an insult to human dignity and rights and that's why UK got this European court of human rights judgment. And now it should comply. But in a typical fashion, the measures amount to almost a disregard to this judgment."

— antonis47

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In Review: Venture Capital, Hot Spots, Salary Surveys Past, Blade Computing

Last year's June issue of *Genome Technology* included a feature story that delved into the world of venture capital in the life sciences. Even a year ago, the marketplace for new life science startup companies was becoming a bit tighter than it had been in the past, though investments were still being made. In the first quarter of 2008, four venture-backed life science companies went public and, according to PricewaterhouseCooper's MoneyTree, $1.08 billion was invested in 132 biotechnology deals during that time. These days, investments are more difficult to come by — for the first quarter of 2009, MoneyTree reports that $577 million was invested in 81 deals. However, Chad Waite, managing director of OVP Venture Partners, told our sister publication *GenomeWeb Daily News* earlier this year that "there is no shortage of good ideas just because the economy is bad."

JUNE 2004

JUNE 2008

In June 2008 we also looked into the hot and the up-and-coming regions for biotech. The established regions included the Boston/Cambridge area, the Bay Area, and North Carolina's Research Triangle, among many other well-known spots. The upstarts on the list were Alabama, with Rick Myers' move to HudsonAlpha; Oslo with its Cancer Cluster, to which the Norwegian government gave expertise status; and China, particularly Shanghai, where Novartis announced a $100 million R&D center. In January, the Norwegian government unveiled a plan to rescue its life science industry, including the Oslo Cancer Cluster. Part of the plan was to triple innovation loans from $44.8 million to $133 million.

2004 marked *GT*'s second annual salary survey, in which 1,180 of our readers participated. Back then, PhD scientists reported that their median income was in the $75,000 to $99,999 range. Three percent of the respondents had been laid off over the course of the preceding year and four percent had suffered a pay cut. For the stats on this year's salary survey, head to page 36.

That year the magazine also spoke with SUNY Buffalo's Jeffrey Skolnick, who was ahead of the curve and replacing his 4,000-processor cluster with a 1.32 teraflop IBM blade system. The blades, he said, allowed for a smaller footprint. Skolnick has since moved to Georgia Tech, where he heads up the Center for the Study of Systems Biology. One of the facilities at the center is a RAZOR cluster, a 1,000-node IBM cluster that can perform 8.5 trillion calculations per second. As of January 2007, the cluster contained 1,154 blades.

—Ciara Curtin

>SHORT READS

Sequenom faces class action lawsuits on behalf of shareholders after the firm announced that it will delay the launch of a test for Down syndrome on discovering that employees had mishandled R&D test data and results. Sequenom CEO Harry Stylli said four employees had been suspended and that the company put a new team in place to oversee studies of its prenatal diagnostics.

John Rossi, co-founder of Dicerna Pharmaceuticals, won the 2008 Cozzarelli Prize from the Proceedings of the National Academy of Sciences' Editorial Board for work using microRNAs for gene silencing.

In a letter signed by businesses, patient advocacy groups, and researchers, the Genetics and Public Policy Center at Johns Hopkins University and the Coalition for 21st Century Medicine asked new Secretary of the Department of Health and Human Services Kathleen Sebelius to implement policy changes that will advance diagnostic and pharmacogenomic technologies. The letter urged Sebelius to strike a balance between patient safety and encouraging innovation when it comes to the oversight of new personalized diagnostics. It also called for a mandatory registry that would include the names of labs performing specific tests as well as the names of the labs or makers that develop tests.

Bioinformatics: Dana-Farber, Quackenbush Launch Analysis Consultancy Center

If the idea of a one-stop informatics shop ready to meet all of your data analysis needs sounds like something for the wish list, think again. Researchers around Boston now have access to just such a service in the Center for Cancer Computational Biology at the Dana-Farber Cancer Institute. The CCCB, which opened its doors in early April, aims to function as a bioinformatics consultancy, offering investigators guidance and services for all types of 'omics research projects. Clients work in tandem with their assigned CCCB consultants throughout the entire analysis process on a fee-for-service basis.

John Quackenbush, director of the CCCB, says that this aspect of collaboration between the clients and consultants is something that will be emphasized, because it's brain power that will solve problems, not just fancy hardware. "A lot of people talk about software and tools to advance or accelerate research, but I think that what is really critical isn't a piece of software, it's a piece of 'gray-ware,'" he says. "And what we hope to do is develop a cohort of people to try and change the way in which people think about analyzing data."

"What is really critical isn't a piece of software, it's a piece of 'gray-ware,'" Quackenbush says.

The new center has its roots in a long-term strategic planning process that started at Dana-Farber more than seven years ago. "They recognized that what was driving modern biological innovation was technology, so they wanted to create a series of centers that worked on an entrepreneurial model to bring in new approaches and technologies and apply them to the study of human cancer," Quackenbush says. "One of the areas they identified early on was bioinformatics and computational biology." Initially the center will be subsidized, but Quackenbush and his CCCB colleagues are hoping to develop a sustainable model over time that might include some institutional support from things like cancer center grants, as well as corporate clients interested in working with the center to help analyze data.

JOHN QUACKENBUSH

"The need that exists for bioinformatics support is underestimated because what these new technologies are doing is converting biological science from a laboratory science into an information science," Quackenbush adds. "And the organizations that are poised to take advantage of the tsunami of data that is coming and on the way already are those that are really going to be able to advance the field."

— *Matthew Dublin*

PHOTO BY SAM OGDEN

Sequencing: Ling Uses Magnetic Field to Slow DNA Strands Flowing Through Nanopore

Sometimes DNA just needs to be slowed down. When the concept of nanopore-based sequencing was developed, the idea was that an electrical field would apply a force to get the strands of DNA to pass through the small nanopore opening while that same electrical field would read the sequence off the strands. "The idea of the nanopore sequencing as originally proposed … is very enticing in its simplicity, that you read off the genetic sequence by measuring current or voltage," says Brown University physicist Xinsheng Sean Ling. "That's very appealing to physicists."

But the electric force needed to get DNA through the pore makes it move too fast for the sequence to be determined; it needed to be slowed down. "The only way to get the DNA into the nanopore is by cranking up voltage, to have a large electric field. And then the large electric field also pushes DNA too quickly. So you can't win. It's a real Catch-22," Ling says.

"For sequencing you need to demonstrate this on multiple pores."

To slow down the movement of DNA through the nanopore, Ling and his graduate student Hongbo Peng, who is now at IBM, decided to separate out the force driving the DNA's movement from the reading mechanism. In their experiment, the motion of the DNA is controlled by a magnetic field, while the reading of the sequence is still done by electrical field. They coated a commercially available magnetic bead, 2.8 microns in diameter, with streptavidin. That streptavidin then attached to the biotin they added to the end of the DNA. As in the original experiments, they drove the DNA through the 10 nanometer-sized nanopore using the electric field. However, the magnetic bead got stuck in the pore. The researchers then used a magnet to pull the bead out of the pore, thus pulling the DNA slowly backward and out of the pore. "The DNA translocates against the electrical field," Ling says. They were able to slow the movement of the DNA by 2,000-fold compared to the original technique.

SEAN LING

There's still a long way to go for this technique to be used for sequencing, such as scaling it up and using different-sized pores. "For sequencing you need to demonstrate this on multiple pores. I think on 10 simultaneously would be very nice. That's not something I'm doing myself here," Ling says. "I'm hoping that a company will pick it up and do that."

— *Ciara Curtin*

>SHORT READS

Paul Billings will be acting director and CSO of the Genomic Medicine Institute at El Camino Hospital in Silicon Valley, where he will help provide genomic medicine services for doctors and patients in a community hospital setting. Billings co-founded GeneSage and CBR Systems, and also worked for Laboratory Corporation of America Holdings.

NHGRI kicked off the second phase of its long-term planning process and is asking the research community to comment on updates made to a series of white papers it released late last year. Comments on the papers — which focus on genomics in clinical practice, the future of genome sequencing, and genomics education — are due June 30.

Last seen as bioscience team leader at Cargill BioTDC, Jose Laplaza is the new director of strain engineering and lab operations at Integrated Genomics.

The Ontario Ministry of Research and Innovation announced that its Global Leadership Round in Genomics and Life Sciences will spend C$100 million to support collaborative research projects in the province. The money comes from the Ontario Research Fund, a C$625 million, four-year effort that is focused on developing research in the region while boosting industry and scientist recruitment.

>SHORT READS

Marco Scarpetta, previously at Orchid Cellmark, was named laboratory director at DNA Diagnostics Center.

Sarah Tishkoff, a geneticist affiliated with the University of Maryland and UPenn, led an international team in what's billed as the largest study to date of African genetics, published in *Science*. The team looked at more than 1,300 polymorphic markers in thousands of individuals from more than 100 African populations, four African-American populations, and 60 non-African populations. The results confirmed relationships between some populations with shared language and culture, but uncovered shared ancestry in other groups that were previously thought to be unrelated.

Peter Collins was promoted to be senior VP of business development at DxS. Collins has held senior executive roles at Vysis Europe, Quantase, Gentronix, Biogenix, and Pronostics.

Amar Sethi, who has worked at NHLBI, will be VP of science and technology at Pacific Biometrics.

The American Civil Liberties Union and other plaintiffs filed suit against Myriad Genetics and others, charging that its BRCA gene patents stifle research and limit options for medical care.

GWAS: New PhenX Toolkit Aims to Make Data Useful to More Researchers

Genome-wide association studies have recently been given a shot in the arm thanks to a free online toolkit courtesy of the National Human Genome Research Institute. A product of NHGRI's three-year Consensus Measures for Phenotypes and eXposures, the PhenK toolkit aims to provide researchers with a grouping of standard measurements for physical characteristics and environmental exposures of research subjects. The impetus for the project arose from a desire to enable researchers to analyze results across a range of research studies.

"We thought if you wanted to actually combine these studies, it would be a shame if you had to use some really limited measure," says NHGRI's Teri Manolio.

"What we're finding is that a lot of these studies, while they may have some really good measures — for example, diabetes — do not always have great measures of cancer or heart disease, or conversely, a breast cancer study can be wonderful on breast cancer, and include histology and slides, but they might be really bad on diabetes measures," says Teri Manolio, director of the office of population genetics at NHGRI, who helps lead the initiative. "So we thought if you wanted to actually combine these studies, it would be a shame if you had to use some really limited measure."

The answer was to arrive at measures that, while perhaps not the gold standard, were valid, useful, and not terribly burdensome. This was easier said than done. "The challenge was really limiting the number of variables that we would look at, so you ask somebody to define metabolic disease or think of all the things we could study in metabolic disease, and they say it's hundreds if not thousands of variables," she says. "But for a study that would be a pooling study or an additional study, you can't measure thousands of variables or thousands of traits, so we decided that we really just need 15, and for most of them, we've been able to get close to that number."

Manolio says another challenge with getting PhenX off the ground was convincing the experts involved to sign off on the finished product, which often required them to make concessions with the way in which particular phenotypic traits might be measured.

The initiative eventually hopes to have 20 health and disease research categories for PhenX, as well as a Facebook-like social networking platform that will enable registered users of the PhenX website to collaborate with other researchers.

— *Matthew Dublin*

Protein analysis: Dinosaur Peptides Intact After 80 Million Years

Using a combination of techniques from immunoblots to mass spectrometry, a group of researchers led by North Carolina State University's Mary Schweitzer confirmed that proteins from Cretaceous-period dinosaurs could be preserved and sequenced. In 2007, Schweitzer and her colleagues had reported on peptides recovered from *Tyrannosaurus rex* remains, though those findings were controversial, as proteins are generally thought to degrade quickly.

For the current project, researchers studied a femur from *Brachylophosaurus canadensis*, a hadrosaur that lived 80 million years ago, to see if it too contained protein remnants. "We basically went looking for a dinosaur preserved deeply in sandstone," Schweitzer says. "If we want the best preservation possible, it's going to come from dinosaurs that have been deeply buried very rapidly after death in sandstone."

MARY SCHWEITZER

Due to the controversy surrounding their earlier *T. rex* study, particularly regarding contaminants, Schweitzer and her colleagues were extra careful in the collection and analysis of *B. candensis*. Instead of excavating the skeleton completely in the field, the researchers took it back to the lab surrounded in six inches' worth of sandstone. "If you think about it, this dinosaur has been sitting in equilibrium with its environment for 80 million years. Then we come in and we dig it up and sweat on it and eat lunch over it and drink beer on it and degradation picks up where it stopped," says Schweitzer. "We wanted to keep it as stable as we could."

When the sample arrived in the lab, the team set to demineralizing it, looking at it under scanning and transmission electron microscopes. They tested to see if hadrosaur tissues and tissue extracts bound to chicken and ostrich collagen antibodies — and they did. Then they analyzed the remains with mass spec, still trying to limit the effects of contamination. "While [one colleague] produced mass spec sequences from extracts of the bone, we also did some *in situ* mass spectrometry and were able to identify amino acid residues that are specific for collagen and localize those to the tissues," she says.

"[We] were able to identify amino acid residues that are specific for collagen and localize those to the tissues."

From those amino acid residues, Schweitzer and her colleagues determined that the collagen sequence from the hadrosaur samples fell in the dinosaur-bird clade. From their phylogenetic tree, they predict that *B. candensis* is more closely related to birds than to alligators and that *T. rex* is more closely related to birds than to *B. candensis*.

— *Ciara Curtin*

>SHORT READS

Case Western Reserve University received a three-year NIDA grant worth up to $3 million to conduct proteomic studies of hepatitis C and HIV infections. Researchers will use the funds to develop proteomic and epigenetic markers for chronic immune activation during HIV disease and for studies of the effects of current and prior drug use and HCV infection on disease progression and therapy.

Barbara Schaal, a biologist at Washington University in St. Louis focused on quantitative genetics and molecular biology-based studies of evolution in plants, is joining the President's Council of Advisors on Science and Technology and will be a science policy advisor for the White House.

PerkinElmer acquired Analytica, which produces electrospray ionization source technology for mass spectrometers, for an undisclosed amount of money.

The Minnesota State Legislature approved $16 million for the Minnesota Partnership for Biotechnology and Medical Genomics.

Helicos BioSciences reported $1.2 million in first-quarter revenues, including its first product revenues at $963,000 from an instrument sale last year, and from reagent sales. The company has installed three systems so far.

Translational research: Study of Protein Linked to Alzheimer's Gives Hope for Therapeutic

In an ongoing quest to find how exactly amyloid β protein is made from the amyloid precursor protein, researchers led by David Kang at the University of California, San Diego, have made a big step in the right direction. In a study published recently in the *Journal of Biological Chemistry*, Kang's team found that Ran-binding protein 9, RanBP9, plays a role by conjugating with another protein to form a scaffold that initiates the enzymatic cleavage of APP to Aβ. The role of RanBP9, says Kang, could make it a possible new therapeutic target for Alzheimer's disease. Current therapeutic targets are major enzymes; because tweaking their production could wreak unexpected havoc in the body, scientists are searching for less dramatic alternatives.

Aβ, which collects as senile plaques in the brains of people with Alzheimer's disease, is generated from APP by two enzymatic cleavages: first by β-secretase (BACE1) and then by γ-secretase (Presenilin). How BACE1 finds APP, though, remained unclear.

"What we found was a protein called RANBP9 that seems to bring these two guys together in scaffolding kind of way," Kang says. "RanBP9 appears to interact with both BACE1 and APP and also another molecule called LRP, and bring these guys together so that the first cleavage can occur."

"What we would like to do is assess whether or not there are some small molecules or compounds that can inhibit this protein."

In previous work, Kang, who is an assistant professor of neurosciences, studied how LRP, or low-density lipoprotein receptor-related protein, can not only remove Aβ by carrying it out of the brain but also stimulate its production. While LRP is a huge protein with a large extracellular domain, Kang narrowed the segment that is needed to generate Aβ to a 37-amino acid stretch within the intracellular domain. "This region had never been studied before," Kang says.

DAVID KANG

In a study last year, Kang used a yeast two-hybrid screen of a mouse cDNA library using the 37-amino acid region as bait, and found four proteins that bound especially well. One of these was RanBP9. In their most recent work, Kang's idea was that perhaps LRP works by recruiting RanBP9. Sure enough, when he expressed it in mammalian cells, he found a "huge increase" in Aβ and when he used RNAi to knock it down in cells, he saw a significant drop in Aβ. "It told us that, in fact, it's normally involved in APP processing and Aβ generation," he says.

Next steps include making knockout animals for RanBP9 and testing in animal models to see if they can confirm their findings *in vivo*. Kang is also convinced of the possibility of RanBP9 as a therapeutic target. "What we would like to do is assess whether or not there are some small molecules or compounds that can inhibit this protein and thereby reduce Aβ production," he says.

— *Jeanene Swanson*

Cancer: Assessing the Role of GWAS in Personalized Medicine

Amid the buzz of the results from the Cancer Genome Atlas and a multitude of studies looking at the epigenomics of cancer at this year's annual meeting of the American Association for Cancer Research, scientists discussed the future of genome-wide association studies. How should scientists take these large data sets and move them forward to translate them into information that is useful for both the diagnosis and treatment of cancer?

At a forum addressing the future of GWAS in personalized medicine, Harvard's David Hunter began by talking about the process of performing a genome-wide association study and the increasingly important role of replication studies. In the discovery phase, the challenge is wrapping your head around all

One-time screening for multiple lower penetrance conditions will be part of the clinical future.

that data, especially if you're not used to a systems biology approach, Hunter said. "Naturally [for] someone who's used to seeing data based on a single SNP, the first challenge is how [to] integrate the data [and] how [to] present it," he said. For the replication phase, Hunter sees a growing need for larger-sized studies to effectively find all the common variants with largest effects in cancer, a process that he thinks scientists should aim to complete in the next couple of years. Despite the concern that association studies are a dead end, he thinks that it's "rational, sensible, and cost-effective to keep doing GWAS while we await the cost-effectiveness of whole genome sequencing."

In the past several years, the number of identified disease susceptibility loci for various cancers has skyrocketed. Culling data from the 2007 AACR meeting, Hunter said that prostate, breast, and colon cancer each had one common variant associated with them; two years later, prostate cancer weighed in with at least 18 variants while breast and colon cancers boast a whopping 40 or more variants each. With that in mind, Hunter believes GWAS will continue to play a serious role in mapping the etiology of cancer, searching for gene-specific mechanisms of diseases, and finding out more about the role of intergenic regions, since many association loci are found within noncoding regions.

While GWAS have increased the number of available risk factors, current risk prediction algorithms are still not reliable enough for clinical use. While Hunter believes that one-time screening for multiple lower penetrance conditions will be part of the clinical future, he also emphasized that scientists must complete the discovery phase for common alleles in common diseases in order to move forward on improving risk prediction. Until the current universe of risk variants is expanded to include all variants, he noted, scientists, physicians, and direct-to-consumer genotyping companies should hold off on considering the information clinically useful.

— *Jeanene Swanson*

Nanopore: Nabsys Sequencing Shoots for Big Picture Instead of Scanning Individual Base Identity

The CEO of Nabsys is not your typical next-gen sequencing technology evangelist. Then again, the Nabsys nanopore technology is not based on your typical sequencing concept.

CEO Barrett Bready studied physics before getting his medical degree, which gives him a distinctly clinical bent when thinking about how nanopore sequencing could be applied. Among his goals: to make sequencing fast, accurate, and cheap enough that clinicians could sequence multiple genomes from a tumor, for instance, in the recognition that cancer patients rarely have completely homogeneous tumors that will respond well to a single course of treatment.

The Nabsys approach uses an electronic measure, rather than the more common nanopore goal of directly reading DNA bases as they flow through. Rather than detecting sequence base by base, the Nabsys concept is to hybridize short probes of known sequence to the DNA strand, feed the product through the nanopore, and then use changes in current to measure where the probes bind and the distance between them. DNA strands could theoretically be longer than 100,000 bases during this process — Nabsys has tested it out on strands as long as 50,000 bases. The probe sequences are then collected and the distances between them fed into an algorithm that overlays the data to generate a full sequence of the DNA in question.

The beauty of the approach, as Bready sees it, is its reliance on solid-state materials for the nanopore rather than the more traditional protein nanopores. This has improved the signal-to-noise ratio by an order of magnitude compared to other technologies, he says, and allows the company to use readily available, time-tested materials from the semiconductor industry.

The company has not sequenced a genome yet, but proof-of-concept studies indicate that a full human genome sequence could be generated at 25x coverage for less than $100 and in less than an hour, Bready says.

Nabsys, based in Providence, RI, was founded in 2004 as a spinout from the physics department at Brown University. The company just completed its first venture round, raising $4 million.

— *Meredith Salisbury*

Of God and Grants

Merck's fake journals, peer review and challenge grants, Francis Collins' new foundation, and the swine flu extravaganza. *By Meredith Salisbury*

Grant Funding & Stimulus

The blogger at **Medical Writing, Editing & Grantsmanship** notes that 18,000 applications came in for challenge grants, with 11,000 more in the error correction queue. The ***Science* Insider** blog determined that with off-the-charts application numbers and the estimated awards available, the success rate may be about 2 percent. Meantime, in a more general post about reviewing grant applications, Steven Salzberg at **Genomics, Evolution, and Pseudoscience** aired his grievance about NIH's password procedures, which require reviewers to log in to the NIH website, get a password for the proposal, and then enter that to view the proposal. "This is ridiculous," Salzberg writes. "Does NIH want us to read the proposals, or not?"

writedit.wordpress.com
blogs.sciencemag.org/scienceinsider
genome.fieldofscience.com

Phony Journals

A blog at ***The Scientist*** noted that Merck published a journal containing reprinted or review articles that reported data favorable to the drug company without disclosing its sponsorship of the publication. The journal — *Australasian Journal of Bone and Joint Medicine* — came from Elsevier, which received an undisclosed sum from Merck for the service. Days later, Jonathan Rochkind at **Bibliographic Wilderness** blogged about an analysis he did indicating that this journal was one of many such marketing materials in disguise. Elsevier's Excerpta Medica Communications label, which printed the Merck journal, ran 50 other publications, all of which Rochkind characterizes as "suspect."

www.the-scientist.com/blog
bibwild.wordpress.com

God, Science, and Collins

Bloggers were engaged in debate about Francis Collins' new BioLogos foundation, established with funding from the John Templeton Foundation, that aims to show people that faith and science can be compatible. Jonathan Eisen at the **Tree of Life** says he perused the foundation's website with "some horror," adding that "science (and medicine) should be about, well, science. And religion can be about whatever it wants to be. … But merging the two together into one hybrid such as Christian Science and Creation Science? Not for me." Over at **Sandwalk**, Larry Moran writes, "Many of us have difficulty understanding how a personal God can be involved in guiding evolution without violating the laws of physics and chemistry."

phylogenomics.blogspot.com
sandwalk.blogspot.com

We Used to Call It Swine Flu

Needless to say, the blogosphere has been fascinated by all things swine flu. Sandra Porter at **Discovering Biology in a Digital World** used it as an opportunity to build phylogenetic trees from different flu strains, finding that "the California swine virus is most closely related to a swine flu virus from Ohio" that occurred at a county fair in 2007. At **Dechronization**, Susan Perkins blogged about different ways that people were tracking the spread of the virus, including a tool called Timemap from Rod Page that traces flu outbreaks on a map. Finally, **Sciencebase** offered a basic Q&A to clear up some confusion over H1N1.

scienceblogs.com/digitalbio
treethinkers.blogspot.com
sciencebase.com/science-blog

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My Take | MICHELLE KIENHOLZ

Better Science with Stimulus

New application format, new funding mechanism, new SROs, new reviewers, new scoring system, new review criteria, new critique format. All in all, an exciting ride for stimulus grants.

With the passage of several of the major grant deadlines for National Institutes of Health programs funded by the American Recovery and Reinvestment Act of 2009 (ARRA), the waiting game begins.

Of course, the funding opportunity drawing the most attention has been the Challenge Grant (RC1) program, which drew more than 20,000 submissions and overwhelmed the Center for Scientific Review. By comparison, CSR handled 27,360 R01 applications in all of 2008. These new applications for two-year grants of up to $1 million were prepared in response to some 900 Challenge Topics — enough to permit the program to be as investigator-initiated as possible while providing NIH a means to track and report the broad areas and more focused topics being addressed with ARRA funding.

As described in the May 2009 issue of *Peer Review Notes*, CSR Director Toni Scarpa opted for a two-stage approach, sending out all applications to at least three reviewers for mail reviews due back June 5. These 60,000-plus critiques will in turn be ranked by CSR and distributed to editorial review board members for preliminary scoring and discussion in July. The 20,000-plus summary statements are targeted for completion in early August, with funding decisions by September.

The $64,000 question is how responsible funding decisions can be made on so many applications in such a compressed time frame, particularly for a new grant mechanism using an untried application format combined with non-traditional criteria and priorities applied during the maiden voyage of a completely revamped review process. While public law dictates that NIH must use scientific peer review in making awards, ARRA-related funding decisions will give weight to additional criteria not usually taken into consideration by our friends in Bethesda.

Different priorities

Indeed, as reported in *The Chronicle of Higher Education*, NIH plans to "tweak its science-based distribution guidelines to ensure the largess some measure of geographic parity." This means spreading the wealth to less well endowed states in the South and Midwest, among others.

Specifically, applicants from states that are eligible for the Institutional Development Award program should have an edge. These states have an NIH success rate (number of applications awarded versus number of applications approved) of less than 20 percent or received less than an average of $120 million per year during the past four years. This select club currently includes Alaska, Arkansas, Delaware, Hawaii, Idaho, Kansas, Kentucky, Louisiana, Maine, Mississippi, Montana, Nebraska, Nevada, New Hampshire, New Mexico, North Dakota, Oklahoma, Puerto Rico, Rhode Island, South Carolina, South Dakota, Vermont, West Virginia, and Wyoming.

A similar program at the National Science Foundation — Experimental Program to Stimulate Competitive Research — adds Alabama, Tennessee, and the US Virgin Islands to the list of regions with funding disparities.

Aside from this unprecedented effort to ensure geographic parity, what other non-science considerations might be taken into account when making funding decisions? The National Center for Minority Health and Health Disparities clearly states that it will only fund proposals that:

- Preserve and create jobs and promote economic recovery
- Assist those most impacted by the recession
- Provide investments to increase economic efficiency by spurring technological advances in science and health

President Obama issued a memo giving the same marching orders to agency heads by recommending funding of projects that will:

- "(i) deliver programmatic results;
- (ii) achieve economic stimulus by optimizing economic activity and the number of jobs created or saved

in relation to the Federal dollars obligated;
- (iii) achieve long-term public benefits …; and
- (iv) satisfy the Recovery Act's transparency and accountability objectives."

In fact, economic stimulus is more than just a good idea. Obama's memo reminds agencies that they "shall not approve or otherwise support any project, application, or applicant for funding that is imprudent or that does not further the job creation, economic recovery, and other purposes of the Act." The president notes a few exceptions: no ARRA funds shall be used for "any casino or other gambling establishment, aquarium, zoo, golf course, or swimming pool."

How many casino-free jobs should NIH applicants propose creating? The more the better, and NIH's Acting Director Ray Kington gave a hint in his testimony before the House Subcommittee on Labor-HHS-Education Appropriations, citing an NIH workforce study showing that "on average, every NIH grant supports six to seven in-part or full scientific jobs."

"A lot is riding on the outcome of this ARRA funding experiment."

A national impact map at recovery.gov estimates how many jobs the folks in Washington anticipate resulting from the infusion of all (not just NIH) ARRA funds. California gains the most at 396,000 jobs, followed by Texas at 269,000 and Florida at 206,000. The state represented by NIH ARRA patron saint Arlen Specter, Pennsylvania, is expected to gain 143,000 jobs, similar to Illinois at 148,000. At the low end of the scale, Alaska, North Dakota, Vermont, and Wyoming are all only projected to gain 8,000 jobs.

Internally, NIH is encouraging its staff to identify projects that meet ARRA objectives, such as creating jobs and distributing funds to diverse geographical areas — and that might be good publicity candidates.

So, applicants should propose to hire new people — not just move people between grants — and spend the money quickly so as to jumpstart not only their science but also the economy.

The NIH must report each quarter how many jobs their awardees have created and how much grant money (i.e., taxpayer dollars) has flowed back into the economy. They will want these statistics to be especially impressive during the latter half of 2009, when most attention will be paid. In any case, with no carry-over and limitations on no-cost extensions, the basic rule will be: spend ARRA funding or lose it.

Unaccustomed to this mentality, many researchers do not realize the importance of a timeline, with specific, measurable milestones laying out how money will be spent each quarter, to their application's success.

Enhanced peer review

Aside from these nontraditional considerations, even the scientific peer review will be a tall order to fill. Reviewers will be examining proposals often in direct competition with their own. They will be looking at just 12 pages of narrative with sections that do not address scientific matters (jobs created, communities benefited, timeline, and milestones). Reviewers will be assessing proposals that came with specific instructions not to include detailed methods and with no requirement for preliminary data. They will be looking at biosketches with just 10 publications and only a page of supporting literature.

Never mind the unfamiliar application format, they will also be using a brand new scoring and critique system. An experienced reviewer can gauge the nuances of a 1.2 versus a 1.8 versus a 2.3 — but will have no experiential basis for separating a 1 from a 2 or a 3 from a 4. The many Challenge Grant reviewers with limited prior experience will have even less of a feel for which proposals should receive what score in each review criteria category.

Because the CSR has been so overwhelmed with RC1 applications, everyone at the NIH with prior experience as a Scientific Review Officer is being asked to pitch in. The CSR has barely had time to instruct its full-time SROs on the enhanced peer review procedures, and these individuals will be the ones guiding reviewers through the process. Fortunately, SROs will be spared any direct backlash: applicants cannot appeal their scores or outcome.

New application format, new funding mechanism, new SROs, new reviewers, new scoring system, new review criteria, new critique format. A responsible way to spend billions of dollars? Time will tell.

Unfortunately, despite such a recipe for disaster, a lot is riding on the outcome of this ARRA funding experiment. Given the transparency that will be enforced, the NIH must show Congress that it can manage taxpayer money successfully and demonstrate results — results that the public can understand in the context of health and disease. Otherwise, policy makers will be less inclined to increase the base appropriation, in which case paylines will drop steeply to historic lows. ■

Michelle Kienholz is a grant writer and research development administrator at the University of Pittsburgh School of Medicine and maintains the blog Medical Writing, Editing & Grantsmanship.

Lab Reunion | THE HARDISON LAB

Data Is Truth

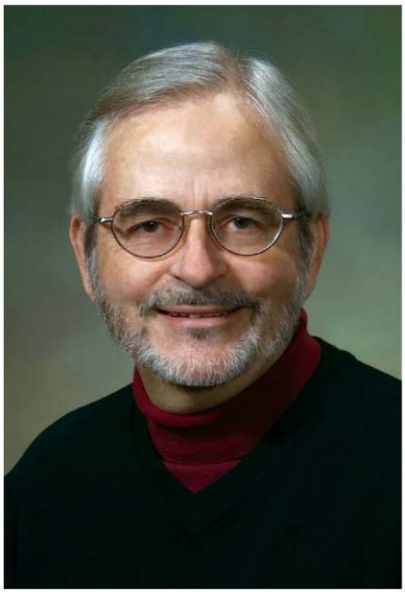
Ross Hardison was at the forefront of genomics before it even had a name. His key to success? Listen to the data and don't get discouraged. *By Matthew Dublin*

It's not often that leading PIs in genomics can trace their current area of focus all the way back to the days before the term was even coined, but such is the case for Ross Hardison. Currently the T. Ming Chu Professor of Biochemistry and Molecular Biology at Pennsylvania State University, Ross and his lab take a comparative genomics approach to predict gene regulatory modules, test them for function in the laboratory, and work to pull all this together into a more thorough understanding of global regulation of erythroid genes.

It all started during his days as a postdoc at Caltech in the late 1970s under the watchful eye of his advisor, molecular and cellular biology pioneer Tom Maniatis. "A lot of great stuff was happening then," Hardison says. "We were trying to isolate eukaryotic genes — that was the big deal in the 1970s — and the techniques that were developed there I think led to the ability to do genomes of complex organisms, like humans."

While Hardison's contributions to genomics are many, worth mentioning is his participation with David Haussler in key human-mouse alignment studies of the International Mouse Genome Sequencing Consortium. Their collaboration demonstrated that the rates of evolution vary markedly along chromosomes and the amount of the human DNA under selection is at least three times the amount of the coding capacity.

In his own career, Hardison has undoubtedly influenced scores of graduate students and postdocs over the years — and he says that that's a result of all the people he was fortunate enough to work with. In particular, though, he points to his graduate advisor, Roger Chalkley, a senior associate dean at Vanderbilt University, and Maniatis as shaping influences. "The thing then with both Roger and Tom was that they were so motivated by the excitement of discovery," Hardison says. "They both had a huge amount of energy and worked real long hours, but there was a lot of excitement which always overrode the frustrations of things that didn't work. When we did discovery stuff it was just great [and] Roger had a really fun lab to work in. … I hope people find my lab fun to work in as well, but it depends upon the personalities of the people in the lab at the time."



ROSS HARDISON

"You try your best to make sure people have the necessary skill sets and they're using them in an optimal way."

Lab coach

Hardison's approach to being a mentor is not unlike that of a sports coach pushing to get top performance out of each player. "You have a group of people in your lab or team and you've got goals to accomplish, and you try your best to make sure people have the necessary skill sets and they're using them in an optimal way," he says. "Every project in my lab, someone is in charge of it — but I'm always saying, 'Oh, you should talk to this other student or check with my research associate because I think this would help.' … Trying to move the projects along as best you can, but everybody has to be engaged and working as a team."

While issues vary from person to person, Hardison says that in general, getting timid students to have self-confidence in the lab is a major

challenge. Oftentimes, it requires a steady hand and a willingness on the part of the students to embrace the research process, which typically includes failure. "There's some people who just walk in and for good reason are very confident, and occasionally you get someone who's awfully self-confident where it isn't necessarily justified," he says. "But much more frequently I get people who are smart and are curious and want to accomplish stuff, but they're just not sure they're doing it right, so you [have] to get them into this mentality of: you just do a plan and execute the experiments well."

He always reminds his students that they have to accept the data as the truth, and that just because an experiment might not yield the desired result, that doesn't make it a waste of time. "There are still some folks who think that the purpose of science is to prove certain ideas and to test hypotheses. … Often people will say, 'I did that experiment [and] the results are bad,' and I say, 'What do you mean by bad?'" Hardison says. "There's no such thing as a bad experiment if you design it well and execute it well, and frequently what they mean is that what we thought was going to happen didn't, but that's fine. The sooner we find it out the better," he adds.

He encourages students to embrace these unexpected or disappointing results and use them as a springboard for future paths of investigation. "Don't fear ruling out an idea because another will come, you should always have multiple models anyway," he says. "Ideas are easy; experiments can be very difficult." Keeping his students energized is a constant priority for Hardison, who says that in good conditions "usually sooner rather than later [students] turn on to this excitement thing."

After spending time under his tutelage, Hardison wants his postdocs and grad students to go out into the world with this as their number one priority: to focus on what is exciting to them — not just what is considered important or what might put them on the map in the research community — without losing sight of the big picture. "That's the advice I give most often, but that's a challenge for some people to do," he says. "I do think we're incredibly blessed that we have money from hard-working taxpayers to do this research and we have a tremendous obligation to society to use these resources well. … But the way you stay engaged is to work on questions that really motivate you and you have a fair bit of control over what you're doing. That's the key to having a happy and exciting career." ■

>NAMING NAMES

The list of folks who have passed through Hardison's lab is a mile long. Here are just a few names you might recognize.

TOM CALLAGHAN

After complete his undergraduate degree in Hardison's lab, Callaghan went on to get a PhD in microbiology from Case Western Reserve University. He was a DNA examiner for the Penn State police, where he was the lead implementer of Pennsylvania's CODIS program. He's been a DNA examiner with the FBI for five years.

LAURA ELNITSKI

After earning her PhD in molecular and cellular biology with Hardison, Elnitski went on to pursue a bioinformatics postdoc with Webb Miller. Elnitski is currently an investigator at the National Human Genome Research Institute, where she focuses on noncoding functional genomic elements.

MARK ROHRBAUGH

After finishing his PhD in biochemistry with Hardison, Rohrbaugh conducted molecular and cell biology research in academic and industrial laboratories. He is currently director of the Office of Technology Transfer at the National Institutes of Health, where he oversees the patenting and licensing of NIH inventions and contributes to intramural and extramural technology transfer policy at NIH and in the US Department of Health and Human Services.

BRAIN SCHEWCHUK

After assisting Hardison with several gene expression studies during his graduate studies, Schewchuk went on to take a position as assistant professor of biochemistry and molecular biology at East Carolina University, where he concentrates his research efforts on chromatin structure modification and epigenetic processes in tumorigenesis.

JAMES TAYLOR

Taylor worked closely with Hardison on several projects, including participating in the Penn State group that provided one of the three major sets of mammalian genome alignments and analysis of patterns of conservation and constraint in several functional classes for the ENCODE consortium. Taylor is now an assistant professor at Emory University.

DAVID VANDENBERGH

During his time in Hardison's lab, Vandenbergh made major contributions to several comparative analysis studies of rabbit genes. He is currently an associate professor of biobehavioral health at Penn State, where he focuses on the control of neuronal gene expression by drugs of abuse and QTL identification of behaviorally relevant genes.

Crossing Disciplines

At the Benaroya Research Institute in Seattle, immunology is the name of the game. Scientists and clinicians have deployed translational research tools to detect biomarkers of transplant compatibility, immune response, and more. *By Jeanene Swanson*

Evolving now for more than half a century, the Benaroya Research Institute at the Virginia Mason Research Center in Seattle was formed in 1956 and then reconstituted itself in 2002 as a center for interdisciplinary and translational medicine. When Director Gerald Nepom came on board in 1985, his idea was prescient: to build it with a "more modern molecular and cellular focus on medicine," Nepom says. While the institute initially focused on immunology and diabetes research, Nepom says it became obvious that the area of autoimmune diseases had become very broad, spanning transplantation, asthma, allergy, inflammation, as well as classic autoimmune disorders like arthritis, diabetes, MS, and lupus. "I guess [the decision to change] was, you could say, driven by the acquisition of scientific knowledge that spurred our thinking that this is really a very broad area that needs an approach that crosses disciplines," Nepom says.

Now Benaroya is a translational immunology center that includes research into the molecular biology and genetics of immune diseases, development of novel and high-throughput ways to study this, and multiple phase I and II clinical trials of new diagnostic tools and therapies. "What's different about us compared to a lot of other immunology places is that we are all under one roof," Nepom says. "All of this is tied together through a series of translational core technologies and laboratories."

Nepom's work revolves around studying the four stages of immune response, which consist of immune cell development, expansion in the circulation, activation at the site of immune stimulation, and counter-regulation that turns the system off at the right time. "What we try to do is figure out how to develop technologies that will measure the activity level at all four stages," he says. "It has major implications for when you treat and what type of immunotherapy is used."

BENAROYA RESEARCH INSTITUTE

Better transplants

Benaroya's Brad Stone started 10 years ago as a postdoc at the institute and now heads his own lab studying minor histocompatibility antigens in bone marrow transplants. He combines genotyping and bioinformatic analysis to predict how donor T cells will respond to novel recipient antigens and whether the recipient will accept or reject the transplant, also known respectively as graft-versus-leukemia and graft-versus-host-disease.

The major and minor histocompatibility complexes are genes that make proteins that present antigens, either foreign or not, to T cells. These genes are highly polymorphic, and T cells have learned to ignore self-peptides and react to foreign antigens. Between two people, the minor histocompatibility genes have many normally occurring polymorphisms that result in a different set of peptide antigens — some of these will be recognized by T cells from donor tissue as foreign. Most of the polymorphisms are either nonsynonymous SNPs or coding deletion

polymorphisms.

"In the first generation of my work, I would use off-the-shelf SNP chips and genotype donors and recipients and compare those genotypes," Stone says. "The goal is to list those alleles unique to the recipient because that defines the protein polymorphisms that the donor T cells have not been tolerized against." After considering what he would do with that information, Stone developed a high-throughput T-cell assay that will tell him which of these polymorphisms will be targeted after the transplant. "The idea that is that it is an unbiased approach, so these SNPs could've occurred in any gene — they could be expressed exclusively in tissues that are targets of graft-versus-host-disease or they could be ubiquitously expressed," he says. "There's basically no bias there as far as tissue expression profile."

His method aims to take some of the uncertainty out of transplantation, in terms of possibility and probability of rejection. Because it's really "sort of a crapshoot for the recipient, the overall goal is to map responses in multiple transplants, and see if there is a hierarchy of minor histocompatibility antigens," Stone says. "Certain types of leukemia … can actually be cured by a bone marrow transplant, and the graft-versus-leukemia response plays a significant role in those patients that are cured. But the flipside is that they risk severe graft-versus-host-disease. Both are caused by T cells from the donor, and both are responding to these polymorphisms that are unique to the recipient."

The first application of his high-throughput T-cell assay would be as a prognostic, for use as a biomarker in the sense of a clinician being able to predict graft-versus-host-disease and be able to, for example, increase immunosuppressive therapy early in the course of the transplant. The longer-term goal is as a therapeutic — the idea is to identify a subset of minor histocompatibility antigens that are only targeted in the graft-versus-leukemia response and "modify the transplant such that we promote the response to the minor [histocompatibility complex] that gives you the benefits that eradicate your cancer, but don't cause a problem in terms of graft-versus-host-disease," Stone says.

Stone thinks that having everybody in one building makes the atmosphere very collegial. What he calls an open-door policy at Benaroya has resulted in several current collaborations, including one with Jane Buckner, director of the translational research program there. He and Buckner are taking genotyping data to find alleles unique to the donor, and then are trying to develop T regulatory cells specific for the donor tissue. "The idea is to use T-reg cells to shut down rejection of that organ," Stone says. In another collaboration with Jay Shendure at the University of Washington, he's just submitted a grant to work on whole exome sequencing of donors and recipients in order to get much more comprehensive lists of all disparate protein polymorphisms. Stone predicts that instead of the 50 percent of polymorphisms that microarrays can find, sequencing will be able to find 95 percent of all nonsynonymous disparities, including frequent SNPs, rare SNPs, and completely unknown SNPs.

>BENAROYA RESEARCH INSTITUTE

Seattle, Washington

DIRECTOR: Gerald Nepom

ESTABLISHED: 1956, renamed in 2002

FACILITY: Located in a single building across the street from the Virginia Mason Research Center; academically affiliated with the University of Washington School of Medicine

STAFF: 220 employees

FUNDING: $24 million a year research volume, local philanthropic gifts

FOCUS: Basic and translational research of autoimmune diseases

CORE LABS: Labs for Sanger sequencing, microarrays, flow cytometry, imaging, and histopathology

Future focus

Benaroya has been at the forefront of probing early-stage immune response for the past decade. Researchers there have developed probes that are now widely used to monitor immune response and to figure out whether the immune system has been activated. Their antigen-specific multi-mer probes can identify and quantify immune cell response. The multi-mer consists of a peptide antigen (which is the immune target and normally is presented to T cells by the MHC protein) bound to an MHC molecule bound in turn to a fluorescent probe. When mixed with blood, the multi-mer acts as a "fluorescent surrogate of the target that T lymphocytes ordinarily see," Nepom says. Typically, he says, the frequency of any antigen-specific T cell in an autoimmune disease is between one in 100,000 and one in 200,000 — that is, too rare to visualize with conventional technologies. In the future, Nepom says the goal is to be able to use the probes for early detection of disease, allowing a clinician to ask, "Is this somebody who's developing a pro-inflammatory phenotype that's predictive of disease, or do they have a regulatory phenotype that indicates that things are under control?" ■

Petascale Coming Down the Pike

Supercomputing is on the cusp of a new era, offering researchers processing power never seen before. Here's a look at machines poised to help the life sciences community chip away at the building blocks of biology. *By Matthew Dublin*

Given the combination of the ever-increasing power of compute hardware and researchers' desire to unlock the mysteries of life, it's no surprise that high-performance computing in the early 21st century is now talking in terms of a whole new scale of computation. While the life sciences community has for some time now been concerned with terrifying amounts of data in terabyte-scale proportions — that's 1,024 gigabytes — there is an even larger scale on the computational horizon: petascale computing. One petabyte is 1,024 terabytes, and to provide some perspective, Google processes an average of about 20 petabytes of data per day.

Gee-whiz factor aside, should petascale and near-petascale systems even be on the radar screen of the life sciences community? So far, there is a resounding yes from many in the molecular simulation research community. "With petaflop-scale performance, [molecular] simulations will reach the time scale of a submillisecond," says Makoto Taiji, a team leader at the RIKEN Yokohama Institute. "This time scale will cover various interesting biological events, including large fluctuations in proteins. … Petascale computing will provide scientific breakthroughs."

Taiji and his team use RIKEN's petaflop-capable supercomputer called MDGRAPE-3 to conduct a range of molecular dynamic simulations, including "post-docking" — a protocol he uses to choose drug candiate compunds with more precision after using the normal molecular docking technique. "We have already found a few successful seed compounds for real drug targets confirmed by the experimental assays," says Taiji. "Their optimization is [underway], and we are trying to use our machine also for the optimizations of the seeds." In the case of MDGRAPE-3, which is not really a programmable machine, porting popular molecular dynamics software to run on its architecture is not that difficult, but it does require a deep knowledge of the software and people power. So far, Taiji says, they do not have enough researchers and programmers to study that many molecular dynamics software packages, although they have managed to port Amber and CHARMM, two popular simulation applications.

RANGER SUPERCOMPUTER, TEXAS ADVANCED COMPUTING CENTER

On the other side of the globe, Nick Grishin, a professor of biophysics at the University of Texas Southwest Medical Center and a Howard Hughes Medical Institute investigator, recently used Ranger, the sixth most powerful supercomputer in the world according to the Top500 list, to solve some very difficult three-dimensional protein folding problems. Housed at the Texas Advanced Computing Center, Ranger came online in February 2008 and Grishin got to use its roughly 62,976 processing cores to help secure top honors in the most recent Critical Assessment of Techniques for Protein Structure Prediction competition, a worldwide contest to predict the structures of a

select number of unknown proteins.

"It's embarrassing to say, but because our algorithms are stochastic, they are not particularly fast to run, and protein chains are very long so it just takes an incredible amount of computer resource to compute those energies," Grishin says. Without Ranger, Grishin says he never would have been able to accomplish the task. "A typical cluster is just too small; it needs to be many more processors. A hundred or 200 processors is clearly not enough for this kind of job. … And the more computations we make, the more likelihood there is that we will hit the right energy function and have something with some medical importance," he says.

Still a rarity

Despite the growing number of petascale machines, it's not as if just anyone can waltz down the hallway of an institute and find one to use. These systems are still relatively rare; there are only two supercomputer sites currently capable of achieving one petaflop peak performance in the US. Los Alamos National Laboratory unveiled its Roadrunner supercomputer only last year, which is listed on the Top500 supercomputing sites list as the world's most powerful supercomputer with a whopping 129,600 processing cores. In late January of this year, the Oak Ridge National Laboratory announced that its Cray XT supercomputer, known as Jaguar, is now capable of a peak performance of 1.6 petaflops. "High-performance computing affects all areas of computational science, including biological research … [and] more and more petascale systems will be coming online," says Jack Dongarra, a professor of electrical engineering and computer science at the University of Tennessee. Dongarra is one of the developers of the LINPACK Benchmark, a series of dense linear equations used to measure a compute system's processing capacity. Dongarra helps run the Top500 list, a semiannual listing of the most powerful computing sites in the world compiled by computer scientists in the US and Germany. According to Dongarra, all high-performance systems will reach petascale in the very near future. "The projections say that all of the Top500 fastest computers will be at petascale in 2015," he says.

"I think petascale computing comes at a very good time for biology, especially genomics, which has to deal with … increasingly large data sets."

More petaflop machines are already on the way. The National Center for Supercomputing Applications has teamed up with IBM to create Blue Waters, a beast of a machine that contains more than 200,000 processing cores and is capable of sustained multi-petaflop performance. Although exact performance figures are still being kept confidential by IBM, all involved claim that Blue Waters will far exceed the performance capabilities of the two formerly mentioned machines by a long shot when it comes online in the summer of 2011.

"I think petascale computing comes at a very good time for biology, especially genomics, which has to deal with … increasingly large data sets trying to do a lot of correlation between the data that's held in several massive datasets," says Thomas Dunning, director of the NCSA at University of Illinois, Urbana-Champaign. "This is the time that biology is now going to need this kind of computing capability — and the good thing is that it's going to be here."

David Bader, a professor of computer science at the Georgia Institute of Technology, has been heavily involved in promoting awareness of petascale computing. In 2006, he co-chaired a workshop that attempted both to lay out a roadmap of recommendations for making petascale computing a reality for the life sciences and also to address its many challenges, the biggest of which is scaling algorithms to run on these mega architectures.

"First and foremost, this is a scale of system that has not been seen before," Bader says. "Just in June, we saw Roadrunner using accelerators like the Cell processor and now we see the Cray XT-5 system at Oak Ridge, so I think that can lead to more experience on how to scale algorithms that can run on all those processors."

In addition to scalability, reliability is another major hurdle. When a system crash causes you to lose a few hundred gigabytes on a simulation or analysis job, that really hurts — but just think of the gnashing of teeth when you're talking terabytes or petabytes of data gone haywire. Efforts such as the Berkeley Lab Checkpoint/Restart project have focused on how to ensure a high level of reliability in these monolithic systems. At the start of the year, the group released a new and improved version of its software, an open-source solution that uses checkpointing to take hourly snapshots of MPI-enabled applications running jobs on large-scale compute systems. The software "works transparently and users do not need to make source code changes to their applications to work with BLCR," says Eric Roman, a member of the Future Technologies Group at Lawrence Berkeley National Laboratory. "On a petascale system with possibly thousands of users and applications, this feature should not be overlooked."

Planning for peta

Given that eco-consciousness now pervades the computing world, supercomputing sites are following suit and are continuing to make moves toward more environmentally friendly infrastructure. For Blue Waters, energy-efficient design is not so much a choice as a necessity. "I think we've gotten to a point where that has to be a priority — where if you don't pay careful attention to that, the power budget can just become overwhelming, so in fact we're at the point right now where that has to be part of the consideration," says NCSA's Dunning. "For example, Blue Waters will be a water-cooled, not air-cooled, machine and the simple reason for that is that it's 40 percent more efficient." NCSA also brought in a specialized team to look at ways to minimize the footprint of the building itself so that most of the power coming into the building is actually used to run the computer rather than all the ancillary things needed for the facility.

Bader also hopes to see petascale computing assisting with big ideas in genomics. "When I think of petascale machines, I think of doing complex operations. So once I can assemble whole genomes and sequence whole genomes and get much richer data sets and combine that with microarray data and other data sources, what I want to be able to do is understand the evolution of whole genomes and compare both the organisms and genes across whole and entire genomes," he says. "And that's a problem that needs both an army of data and also the computational requirements of a petascale system. So rather than just taking our current techniques and running them a bit faster, I think that developing new algorithms ... is really where we're headed."

And Bader reminds researchers that this isn't something only computer scientists should be thinking about. "Biologists will need to be aware of this technology because if you push out the road map 10 years, these are the capability class machines that they'll have in their laboratories," Bader says. "There are a lot of biological problems that are still in their infancy and we [now understand] to solve those problems we'll have to bring in a lot of data collected from a lot of sites and a lot of laboratories. … [There will] be a growing number [of biologists] who need access to massive volumes of data and the computational capabilities to solve their particular scientific inquiry." ■

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EPIGENOMICS

A Global View of Methylation

Thanks to microarrays and sequencing, it's finally possible to interrogate methylation profiles on a genome-wide level. Common diseases are among the early beneficiaries, and cancer is in the lead.

BY JEANENE SWANSON

MARTIN HIRST

Years after the high-quality draft of the human reference genome was delivered, it's now well known that it's not just genetic variation that causes differences in gene expression. Epigenomic changes, whether DNA methylation or histone modifications, are increasingly being studied for their role in both normal and disease-associated phenotype changes. The NIH-supported Roadmap Epigenomics Program, an international effort to create reference epigenomes for a variety of cell types, aims to do for epigenetics what the Human Genome Project did for genomics and the HapMap Project did for genetic variation. Ultimately, using these maps to study the relationship between epigenetic changes and disease will allow scientists to home in on how cancers and common diseases develop.

Hot on the heels of the discovery push has been technology development. Thanks to microarrays and next-generation sequencing, new methods let scientists profile methylation on the genome-wide level, and not just at a handful of CpG sites. "We're really at that stage of defining what [the] reference methylation state is, and then from there we can start to investigate how much variation there may be," says Martin Hirst of the British Columbia Cancer Agency. "To do that, you obviously need to do it genome-wide and at the highest resolution that you possibly can."

MD Anderson's Jean-Pierre Issa, who studies methylation in aging and cancer, adds that the idea behind reference epigenomes is that "we need to know what is normal, and then we can figure out what is abnormal."

Methods

Arrays, while still used as a readout technique, are becoming a thing of the past with the advent of accessible next-gen sequencing tools. For some time, people have been using microarrays to profile methylation on CpG islands, and several vendors offer genome-wide arrays. "The current belief is that only DNA methylation in the context of CpG [sites] is biologically meaningful," says University College London's Stephan Beck, formerly co-leader of the Human Epigenome Project and now advisor to the much larger NIH epigenomics roadmap. "This might change, but this is what our current understanding is."

In cancer, for instance, it's well known that CpG islands in the promoter regions of tumor suppressor genes, which are typically unmethylated, become methylated and thereby turn on aberrant gene expression. However, focusing solely on CpG islands doesn't really give a genome-wide look. "It represents a collection of known CpG islands, but it doesn't include any intergenic regions or other regions that may be methylated," BCCA's Hirst says. "And some of those methylations undoubtedly have biological relevance."

The move toward genome-wide profiling includes techniques such as using methylation-sensitive restriction enzymes, bisulfite conversion, and affinity capture. Generally speaking, Issa says, "they usually rely on ligation of some adaptor to a site that is either methylated or unmethylated, PCR, and then microarrays or more recently, sequencing."

In restriction enzyme-based approaches, a methyl restriction enzyme

can be used to cut unmethylated DNA but not methylated DNA, followed by shotgun sequencing of that fraction. According to Issa, "The advantage of that is that all you need is the DNA and the restriction enzyme. The disadvantage is that you are looking only at the restriction enzyme site," which is typically one or two CpGs in a CpG island which consists of 20 or 30 or more, "so you are limited in your resolution." Because CpG islands tend to behave in the same way when it comes to methylation status, it's a good way to get a snapshot with a fairly high degree of accuracy even though the actual coverage of CpG sites is relatively low. Hirst says the disadvantage of restriction libraries is that regions that don't have those enzymes won't be represented.

In affinity capture methods, such as MeDIP, an antibody to methylated cytosine is used to immunoprecipitate the methylated portion of the genome, which is followed by sequencing. "It's pretty good for genome coverage, [but] it's lower resolution than some other methods," says Joe Costello at the University of California, San Francisco, adding that resolution is around 100 to 300 base pairs, rather than a single CpG site. Costello says that using a methyl restriction enzyme and MeDIP on the same sample works well. "One of the advantages is that it's pretty comprehensive and it doesn't require as much sequencing, [in other words] lower cost," he notes.

Jin Billy Li, a postdoc in George Church's lab, says, "This method is often biased toward CpG islands, or the regions with more than one or two methylated cytosines." Hirst adds that the disadvantage of MeDIP is that repeat sequences tend to be overrepresented.

Bisulfite conversion is also widely used, and many think that this approach would ultimately be the gold standard. While bisulfite sequencing has only been applied to plants — Joe Ecker at the Salk Institute performed a single base-pair resolution analysis of DNA methylation in *Arabidopsis* using bisulfite conversion followed by whole genome shotgun sequencing in 2006 — it's far too costly to do for a large mammalian genome. Treatment of DNA with bisulfite converts cytosines to uracils, but leaves methylated cytosines alone. Subsequent PCR or sequencing can tell the difference between the bases. "The major advantage and the reason why bisulfite methods are the gold standard, whether you're looking at a single gene or genome-wide, is that every time you come across a CpG site, you get a yes or a no" as to whether it's methylated, says Costello.

JEAN-PIERRE ISSA

One disadvantage to bisulfite conversion followed by shotgun sequencing is the cost. Also, Issa says, "The disadvantage of bisulfite is primarily that the chemical treatment really degrades DNA down to a pretty low level, often down to 200 or 300 bases. This therefore limits what one can do." As an alternative, the Broad Institute's Alex Meissner led development of a method called reduced representation bisulfite sequencing, or RRBS, where one uses a restriction enzyme to reduce the size of the DNA sample to a small, but targeted, portion of the genome. That's then treated with bisulfite and sequenced. Using the MspI restriction enzyme and a chosen fragment size of 300 base pairs, "it will give [you] a lot of CpG islands which tend to be near promoters, but also a significant subset of fragments that are well outside of CpG islands," Costello says, "so it is biased to a certain part of the genome, but it certainly represents a lot more than just that part."

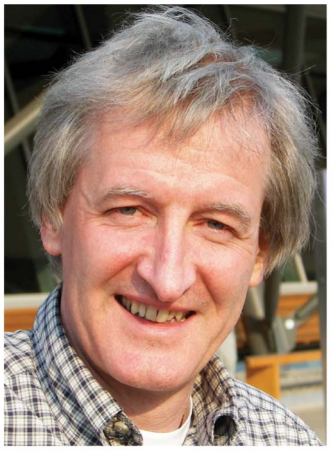
As one of the four labs awarded Reference Epigenome Mapping Center grants as part of the NIH roadmap, Hirst's group at BCCA is still investigating what works best. Issa thinks that bisulfite methods and, eventually, bisulfite sequencing will become the gold standard, possibly even in the next six months to a year. "I think the jury is still out of which is the best and it may be that there's some combination of those methods that's going to be required to actually comprehensively profile the methylated genome," Hirst says. "It's probably likely that each will have [its] own bias, to some degree."

In disease

The goal of the mapping centers is to categorize the normal methylation and histone mark profiles — scientists are limited to studying these with chromatin immunoprecipitation right now — so that they can serve as references. While common diseases in general will eventually benefit, cancer is front and center. There is a well-known link between genome methylation and cancer, specifically that not only do CpG islands in the promoters of tumor-suppressor genes become hypermethylated, but also there is genome-wide hypo-

methylation as the tumor progresses. Hirst says, "Understanding the consequences and causes of global hypomethylation in tumor progression is of great interest" to his lab, and he's studying methylation patterns in stem cells as a model system for tumorigenesis.

While the NIH roadmap project is finding normal patterns of methylation and histone modifications, Stephan Beck's lab at UCL is one of many busily profiling the cancer methylome as part of the International Cancer Genome Consortium, which aims to obtain a comprehensive description of genomic, transcriptomic, and epigenomic changes in 50 different tumor types. "What the majority of people now believe is that there are more epigenetic changes in a cancer genome than genetic changes," Beck says. "The difficulty is to tease out the driver mutations from the passenger mutations, basically the mutations that cause cancer rather than those that are a consequence of the cancer."



STEPHAN BECK

JIN BILLY LI

To accomplish this, he performs global methylation analysis on cancer tissue to find out exactly where in the genome methylation changes occur. "Is it random?" he asks. "Is there anything we can see which helps us understand how the mechanism and how the timing of these changes is coming about?"

While there's less data available, many people think methylation might be relevant for a whole host of things — for example, common diseases, stem-ness and differentiation, brain function, and more. "Really, name the disease and people are interested in whether there could be an epigenetic component to it, and whether it could be detected by methylation," says Issa at MD Anderson.

For common diseases, Beck says GWAS are not enough to explain what causes a certain phenotype. To that end, he's begun incorporating methylation analysis into GWAS, looking for changes which he then ties across cases and controls. While GWAS for genetic changes have a good four- to five-year head start on epigenetics, integrating the two lets him look for what he calls "hepitypes" (haplotype-epitype) in common diseases. "These, we believe, have higher chance of being causal than consequential," Beck adds.

Right now, Issa says, most clinical application of all this epigenetic typing has been at the level of single genes or small panels of genes, where people are looking for methylation as an indicator of the presence of cancer in blood, or in the relationship to disease prognosis or response to therapy. "But whether whole-genome analysis in every single case of cancer, for example, is going to help as opposed to just studying a few genes remains to be seen," he says.

On the horizon

While bisulfite sequencing may be the gold standard, truly affordable, next-gen whole-genome shotgun approaches aren't here yet. RRBS is one method to capture a targeted portion of the methylome, but there are others that are equally promising. Two complementary papers published recently in *Nature Biotechnology* used padlock probes to capture a subset of the genome. In the first, led by Kun Zhang and Virginia Commonwealth University's Yuan Gao, they designed about 30,000 probes that allowed them to look at genome-wide methylation across CpG sites on three chromosomes. Gao's goal was to "specifically target a certain region of the genome in a single tube without doing many, many PCRs," and in this paper, they proved that it could be used with bisulfite sequencing. George Church's lab did similar work, using both padlock probes and a technique called methyl-sensitive cut counting, which cuts the DNA into probes and ligates them to create a library of fragments of relatively uniform size. Co-author Jin Billy Li adds, "One of the main features is the high specificity" to the portion of the genome that was actually targeted, and he sees capturing methods becoming even more targeted in the future.

Nanopore sequencing is another "next-next gen" method for doing global profiling. The change in the current through the nanopore as a single DNA molecule passes through permits a direct reading of the DNA, and this technique would be able to tell methylated from unmethylated cytosine residues. "The beauty of this system is that it will be able to analyze methylated DNA as you isolate it from the cell," Beck says. "That means without bisulfite conversion, without enrichment, without labeling" for unbiased profiling at every single CpG site.

"If that ever works, that's the future of this analysis," Issa says. ■

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GENE EXPRESSION

qPCR Grows Up

It's easier, faster, and more robust than ever. For qPCR, that's both the advantage and the disadvantage. Researchers have developed standards for qPCR experiments, which are increasingly important as the tool gains a clinical impact.

BY CIARA CURTIN

It's been 26 years and one Nobel prize since the light bulb went off over Kary Mullis' head to usher in the age of PCR. An offshoot of that research-changing approach, quantitative PCR, is reaching its stride in its own right. qPCR has grown up and come into its own as a gold standard in quantifying gene expression.

In the decade or so since qPCR was introduced, it has been streamlined and has become a mainstay in many molecular biology labs. "It is very fast, cost-efficient, and easy to handle, while being a very reliable and sensitive method," says Soroush Sharbati from the Freie Universitaet Berlin. "The development of fast and space-saving cyclers and dropping cost prices contribute to the fact that qPCR is widespread in the molecular biology field."

Quantitative, or real-time, PCR keeps getting better with tweaks to its chemistry to make it faster, programs to help pick better primers, and simplifications to the procedure. Though it is commonly considered the gold standard for gene expression quantification, qPCR still suffers from a lack of proper normalization techniques and standardization. Now, however, researchers and companies are calling for certain minimum standards to be followed in qPCR experiments and are also looking for ways for qPCR data — as the pile keeps growing taller with all the multiplexing — to be more easily shared amongst researchers. Despite those drawbacks, qPCR is just getting started, and its next target is the clinic. There, researchers hope that it can be applied to diagnose disease and even stratify patients for more directed therapy.

JEREMY GARSON

"We used various PCR techniques prior to the real-time and they were a big advance on what had gone before, but the real-time version is much more reliable, accurate, and reproducible. It has so many advantages," says Jeremy Garson at the University College London Medical School. "The dynamic range is extremely broad for real-time, and that's particularly useful for virus detection and quantification because the range of concentrations that you get in patients ranges over many orders of magnitude."

qPCR is a-changin'

Compared to the early days, qPCR is much easier to use now. Instead of creating their own master mixes, choosing primers half-blindly, and opening up their tubes halfway through the reaction, researchers can now buy reagents off the shelf and use a software program to help decide what primers are the proper ones to use, while the process has become automated. "I guess it's fair to say it's just that much easier now," says Jon Sherlock, a product manager of TaqMan Arrays and Gene Signature Plates for Applied Biosystems Genomic Assays at Life Technologies. "We have more than 1.2 million pre-designed assays. People can just pick and choose from off-the-shelf reagents." With prêt-à-porter reagents, uniformity and robustness are added benefits.

Roche's vice president of global research, Walter Koch, agrees, adding that scientists have more options these days. "That makes it a lot easier to set up new assays and run them together without having to spend as much time optimizing each and every assay like we used to have to do in the past," he says.

The chemistry of those reagents has also improved and gotten faster — reactions take half the time that they used to. According to Sherlock, the changes to the enzymes, probes, and quenchers all work together to make the qPCR reaction a faster and more robust one. Richard Kurtz, Bio-Rad Laboratories' amplification marketing manager, adds that the fast chemistry reagents have changed the game dramatically. Bio-Rad recently launched Supermix, one of its next-generation reagents. Kurtz says it's significantly faster as it decreases the time of the annealing and extension stages. What normally would take 15 to 30 seconds is now done in two to five.

"There is a clear trend for going to ever decreasing reaction sizes and ever decreasing run times as well."

Jo Vandesompele
GHENT UNIVERSITY HOSPITAL

Fast chemistry has been changing the reaction across the board. "Another big evolution has been the use of fast chemistry — being able to do the reactions in a fraction of the time and decreasing time to results, increasing throughput," Sherlock says.

The throughput hasn't begun to hit its limit, adds Koch. Well plates have evolved from 24, 96, 384, and now are approaching 1,536. "There's nanoscale opportunities for companies like Fluidigm and others that can potentially go another order of magnitude higher," he says, adding that companies can automate so that a single sample can have a number of reactions run from it. For the researchers, access to bigger plates means using less sample and reagents while increasing sensitivity.

"There is a clear trend for going to ever decreasing reaction sizes and ever decreasing run times as well," says Ghent University Hospital's Jo Vandesompele.

At the same time, qPCR reactions have benefited from better knowledge and annotation of the biology. Primer design software can aid the search for specific primers and many of the programs, such as Vandensompele's RTPrimerDB, are readily available online. "It's the upfront knowledge that has increased specificity, not the actual qPCR reaction itself," says Sherlock. He adds that though there have been improvements to instrumentation and optics as well as tweaks to the chemistry, "in essence, the qPCR reaction hasn't actually changed in all this time." Instead, the researchers' expertise has changed. For example, they now know more about the sequence they are designing primers against, which helps them avoid erroneous hybridization.

It's certainly a check in the 'pro' column that qPCR has remained straightforward to use. The disadvantage that some researchers have become increasingly vocal about is that qPCR can seem deceptively simple.

Living up to standards

Being straightforward to use is a blessing and a curse. Because it's PCR, everybody knows the basics — you just need your target, your primers, and some master mix to throw in the machine. However, the intricacies of qPCR can be overlooked.

Stephen Bustin from Barts and the London School of Medicine and Dentistry says that unless standards are followed faithfully, data can be used to show anything. To highlight that, he points to the now-disproven studies from Andrew Wakefield and his colleagues that showed an erroneous link between the MMR vaccine and autism. "I was so angry about the way the RT-qPCR data had been applied to try to link the MMR vaccine with measles and autism that I felt we really need to make a stand here and make people aware of the fact that this can't go on the way it's been going on," he says. Bustin is now at the forefront of a movement to get researchers to follow a set of guidelines, the minimum information for publication of quantitative real-time PCR experiments, or MIQE, that were published online at *Clinical Chemistry* in February.

"In my talks, I always refer to the cowboy stage of qPCR. For quite a while everything went," Bustin says. In particular, he casts a critical eye on how people have been normalizing their gene expression data. In northern blot and standard PCR experiments that didn't give quantitative data, people often used a single reference gene. "People just moved that approach to qPCR without thinking about what they were doing," Bustin says. "Are these reference genes really invariant or are they changing with treatment?"

The MIQE guidelines ask researchers to think their experiments through and to be as transparent as possible. The checklist says that essential information, such as the name of the kit used for DNA extraction, the complete reaction condition, and qPCR analysis program, should be included when the study is published. Other information should be included if known, such as volume of the samples, evidence for optimization, and power analysis. Disclosing

the probe sequence is "strongly encouraged" though the authors note this is not always possible as some vendors do not provide that information to users.

Another effort for standards and transparency in qPCR experiments is taking on the data format. Many researchers cannot look at their colleagues' data because they use different instruments and analysis software. Ghent's Vandesompele says that a universal data exchange format is sorely needed. "Almost 40,000 papers in the biomedical literature are using real-time quantitative PCR and it's ever increasing. It's almost as exponential as the PCR reaction itself," Vandesompele says. "The problem is that we cannot analyze or reanalyze our collaborators' or our colleagues' or peers' work." To go along with the MIQE guidelines, they've come up with a universal data format called RDML that will allow researchers to share their results and "speak the same language." That language and reporting guidelines are in the April issue of *Nucleic Acid Research*.

Target: clinic

Despite the issues surrounding normalization and standardization of qPCR experiments, the technology is marching headlong into the clinic. "For pathogen diagnosis, it is valid, if done properly," Bustin says.

The demand from the clinic is on the rise. "We are seeing more real-time PCR being used in the clinical diagnostic setting," Bio-Rad's Kurtz says.

Already, qPCR is hard at work diagnosing viruses. In April, the US Food and Drug Administration issued an emergency approval for a molecular diagnostic assay to identify cases of H1N1 swine flu — and the approach used was real-time PCR. The test used is an rRT-PCR Swine Flu Panel with a CDC assay and runs on Applied Biosystems' real-time PCR machine. "The Applied Biosystems 7500 Fast and Fast Dx real-time instruments have been authorized by the FDA for emergency use in diagnosing swine influenza A using the CDC's specified test at the CDC-qualified laboratories," Sherlock says. "Clearly there's so much confidence in qPCR now that scientists can move beyond the research labs into regulated environments."

STEPHEN BUSTIN

The same approach was also used in England. At the end of April, Jeremy Garson said that three positive cases of swine flu had been identified in England using a real-time approach from a routine flu assay. Garson often uses qPCR, replacing tissue culture and immunofluorescence, to identify respiratory viruses including influenza A and B but also RSV, metapneumovirus, parainfluenza type I, II, and III, and adenovirus. A few years ago he used qPCR to identify SARS. "It's quick and relatively easy to develop and introduce new assays once the expertise is present in the laboratory," Garson says.

His focus isn't limited to respiratory disease; Garson also looks at other viruses, particularly in bone marrow transplant patients, hepatitis patients, and HIV patients. "One of the key roles is in monitoring antiviral efficacy, " Garson says. "We can determine if [the patients] are non-responders or sustained responders or transient responders and we can modify therapy according to the response."

For some new assays, there are not always standards against which to compare or to determine the significance of a patient's viral level — unlike HIV and hepatitis B and C, for which there are international and national quantification standards. "In some situations, we don't yet know the clinical implications of a certain level," Garson says. "Because the assays are so sensitive, there is the danger of worrying the patient unnecessarily unless quantitative results are available. In most instances, real-time qPCR allows us to discriminate confidently between clinically insignificant low levels of virus and potentially serious higher levels."

Academicians aren't the only ones eyeing the clinic. Roche's Koch says they are interested in moving qPCR into oncology. "I don't think we've begun to exploit … all the ways you can use it to guide differential diagnosis as well as therapy selection," he says. Last year, he says, the big news was that the KRAS mutation could help guide EGFR therapy; he notes that there's a "host of genes that are commonly mutated in common cancers that impact how a patient is going to respond to therapy." Now, he adds, it's practical to stratify patients and optimize therapy.

The future of qPCR most likely holds more of the same as its recent history. "More, faster, quicker, better," says Life Technologies' Sherlock.

Bustin adds that due to all its advantages, the number of qPCR papers is growing exponentially. "I'm very confident about the future of qPCR for the next five years, certainly, and probably longer," he says. "I think that's why it's important that we start getting our act together." ■

COMPENSATION

The 7th Annual Salary Survey

Are you paid as well as your peers? Find out about salary by job title and region, plus information on layoffs, raises, and grant funding.

BY MEREDITH W. SALISBURY

With the global economic crisis and rising unemployment rates, you may feel it's enough just to have a job. But even in this kind of economy, basic human nature — and therefore the need to find out if you're being paid what you're worth — goes on.

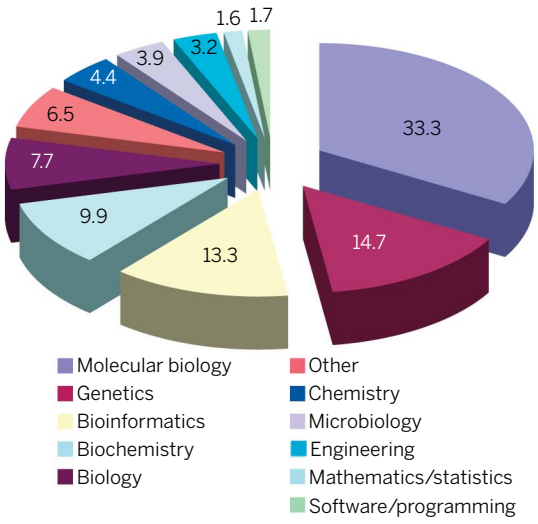
This spring, *Genome Technology* conducted its seventh annual salary survey, and 1,468 readers weighed in with information on their compensation, benefits, and expectations for the near future. This year for the first time we included a set of questions specifically for people who are currently unemployed, and we asked about readers' plans for applying for stimulus grant funding. As usual, we begin the survey results with plenty of demographic data so you can get a sense of who our typical respondents are.

With three percent of respondents reporting themselves as unemployed and another two percent saying that they'd been laid off in the past year but had found new work, it seems clear that layoffs in this community have risen but are significantly lower than in many other industries.

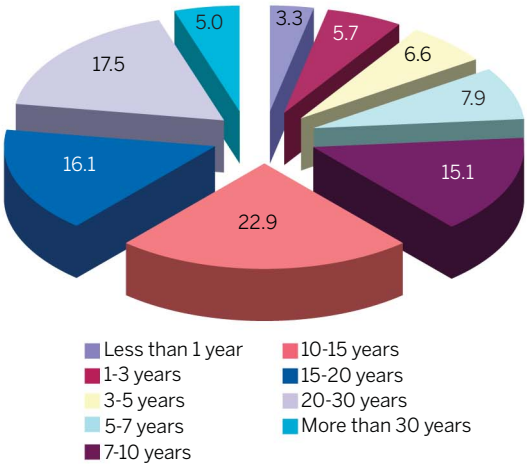
After hearing anecdotal evidence that jobs have evolved because of economic conditions, we asked readers to report on how their jobs have changed in the past year. Data show that scientists have responded to changing situations by attending fewer conferences, applying for more grants, taking on more responsibilities, and working longer hours. A much smaller group of people reported having to reduce staff or put hiring plans on hold.

To conduct this survey, *GT* emailed a link to the survey website to subscribers and sent out a reminder email several days later. The survey data was gathered in late April.

Primary Scientific Backgrounds (%)



Years in Research (%)

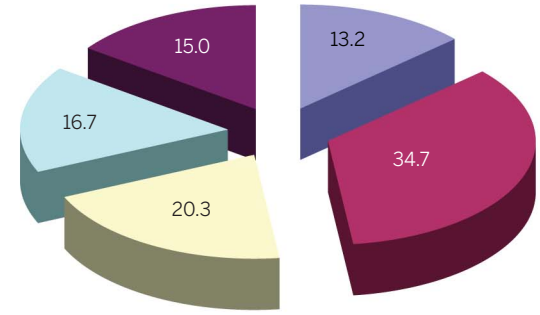


CHARTS BY KEVIN HAND FOR GENOME TECHNOLOGY

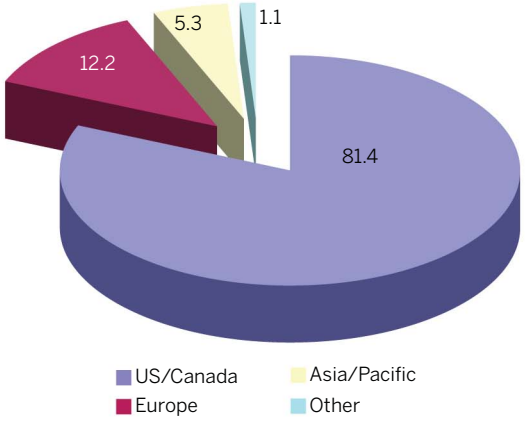
Number of Respondents: 1,468

NOTE: PIE CHART DATA BEGINS TOP CENTER AND GOES CLOCKWISE

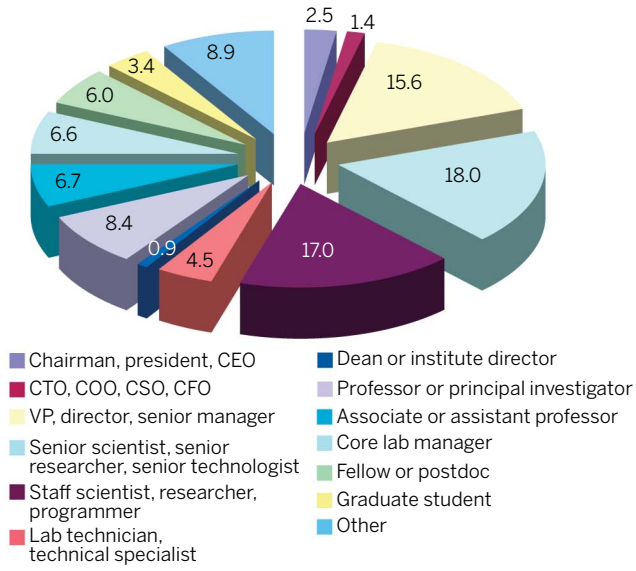
Years in Current Job (%)



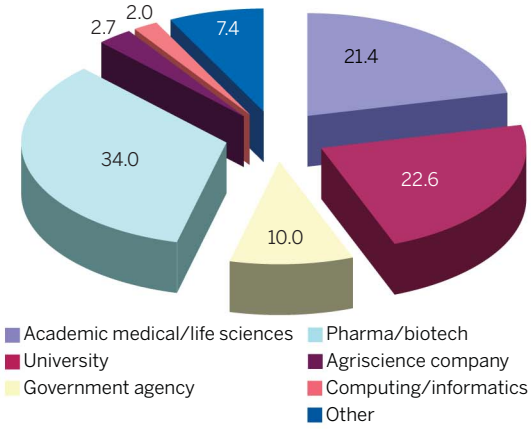
Regions (%)



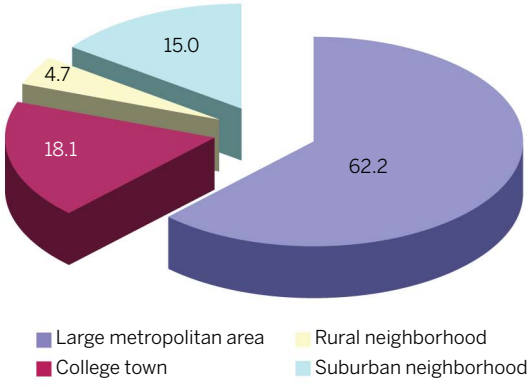
Job Title (%)



Type of Organization (%)



Type of Area (%)



Datapoints

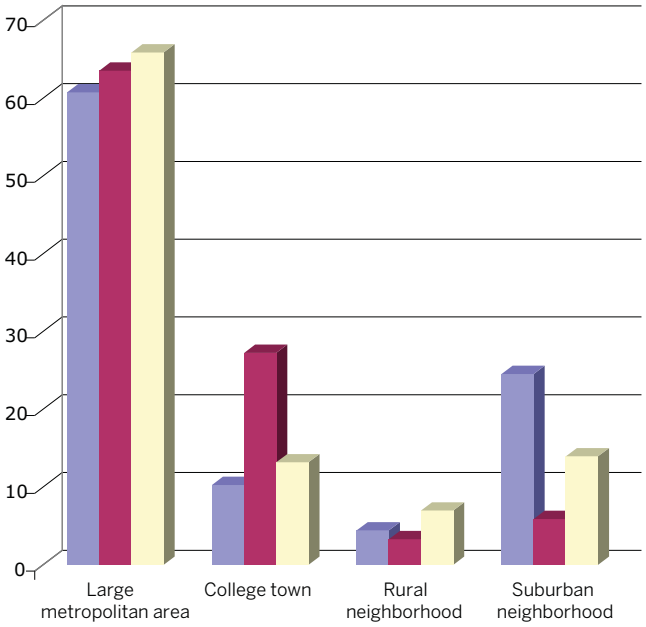
23% OF ACADEMIC RESPONDENTS HAVE TENURE

9% OF RESPONDENTS SAY THEIR LAST EMPLOYER IS NO LONGER IN BUSINESS

42% OF RESPONDENTS SAY THEIR ORGANIZATION HAS HAD LAYOFFS IN THE PAST YEAR

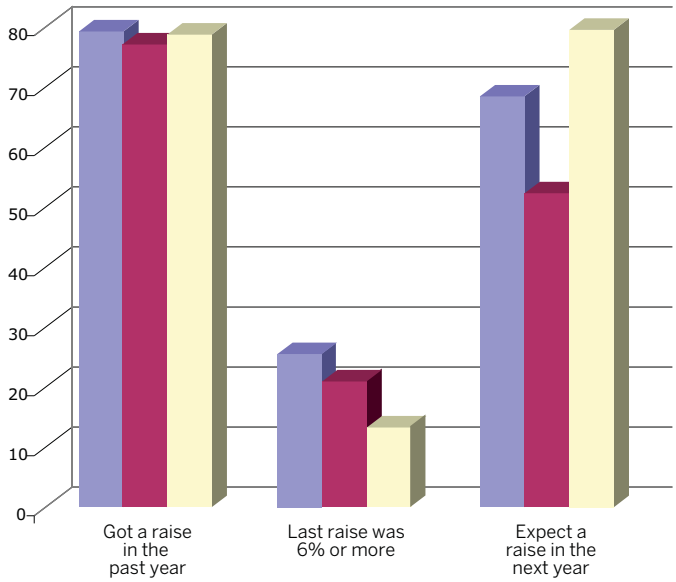
6% OF RESPONDENTS HAVE SUFFERED A PAY CUT IN THE LAST YEAR

Type of Region by Organization



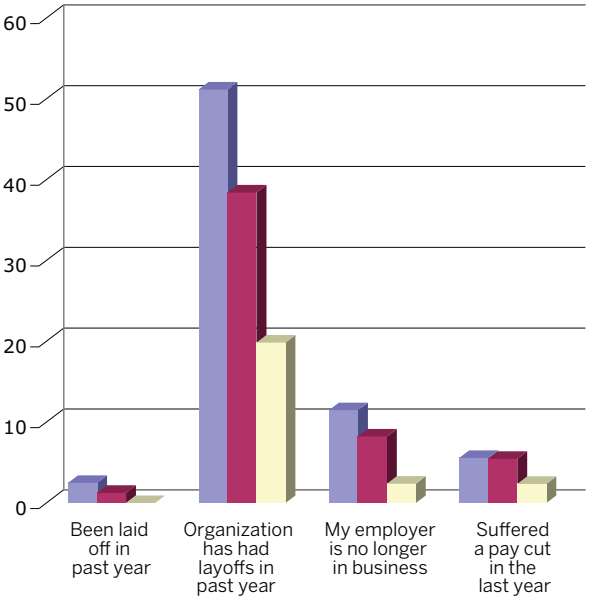
Percent of respondents in each type of organization:
Pharma/Biotech Academic Government

Raises Expected by Organization



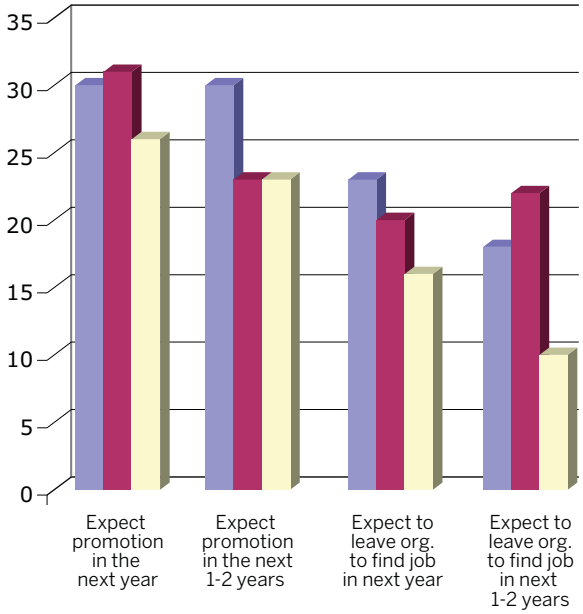
Percent of respondents in each type of organization:
Pharma/Biotech Academic Government

Layoffs and Pay Cuts by Organization



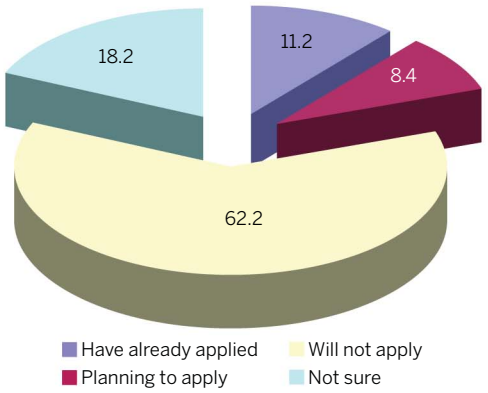
Percent of respondents in each type of organization:
Pharma/Biotech Academic Government

Moving On and Up by Organization

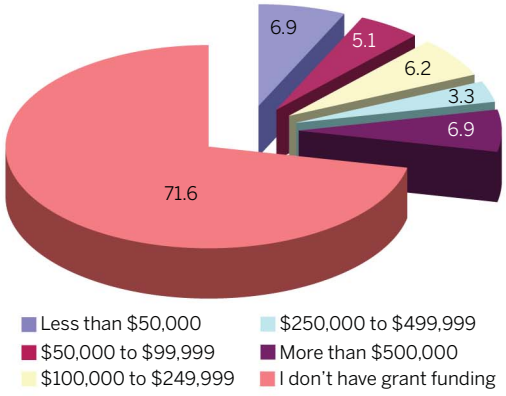


Percent of respondents in each type of organization:
Pharma/Biotech Academic Government

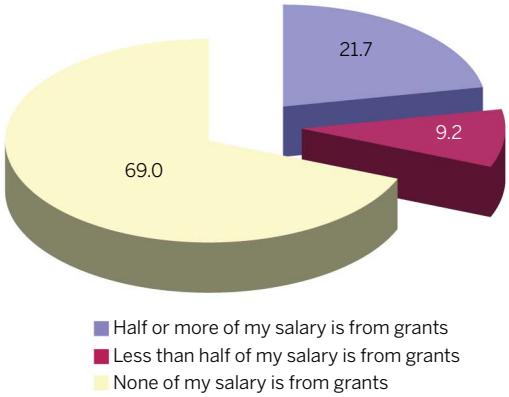
Stimulus Funding (%)



Grant Funding Awarded (%)



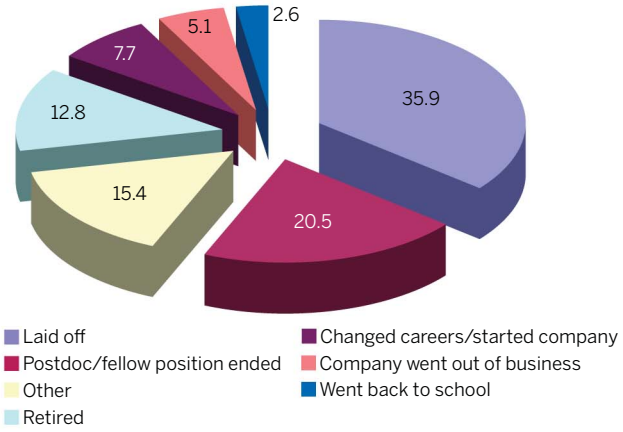
Salary from Grant Funding (%)



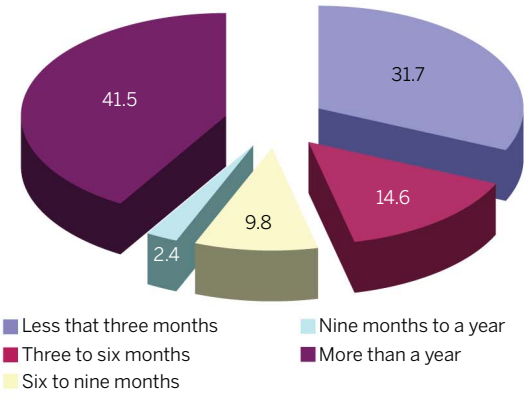
Results from Unemployed Respondents

NOTE: PIE CHART DATA BEGINS TOP CENTER AND GOES CLOCKWISE

Reason for Leaving Last Job (%)



Length of Unemployment (%)



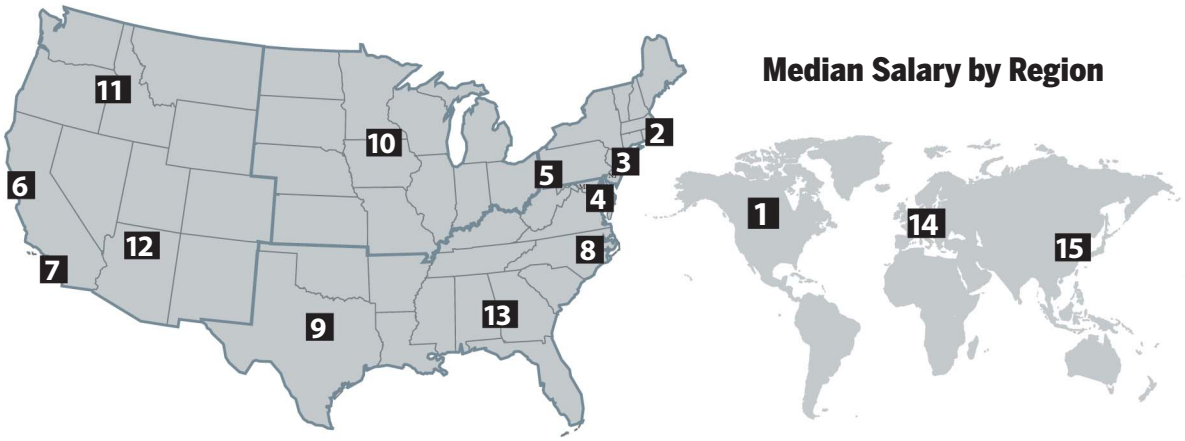
Most Common Tools Used in Job Search

1. Career pages on company/institute websites
2. Social networking sites
3. Trade-specific websites
4. Contacting colleagues and friends

Datapoints

3.17% OF RESPONDENTS ARE NOT CURRENTLY EMPLOYED

51.4% OF UNEMPLOYED RESPONDENTS ARE ACTIVELY SEEKING JOBS


Median Salary by Region
1
2
3
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5
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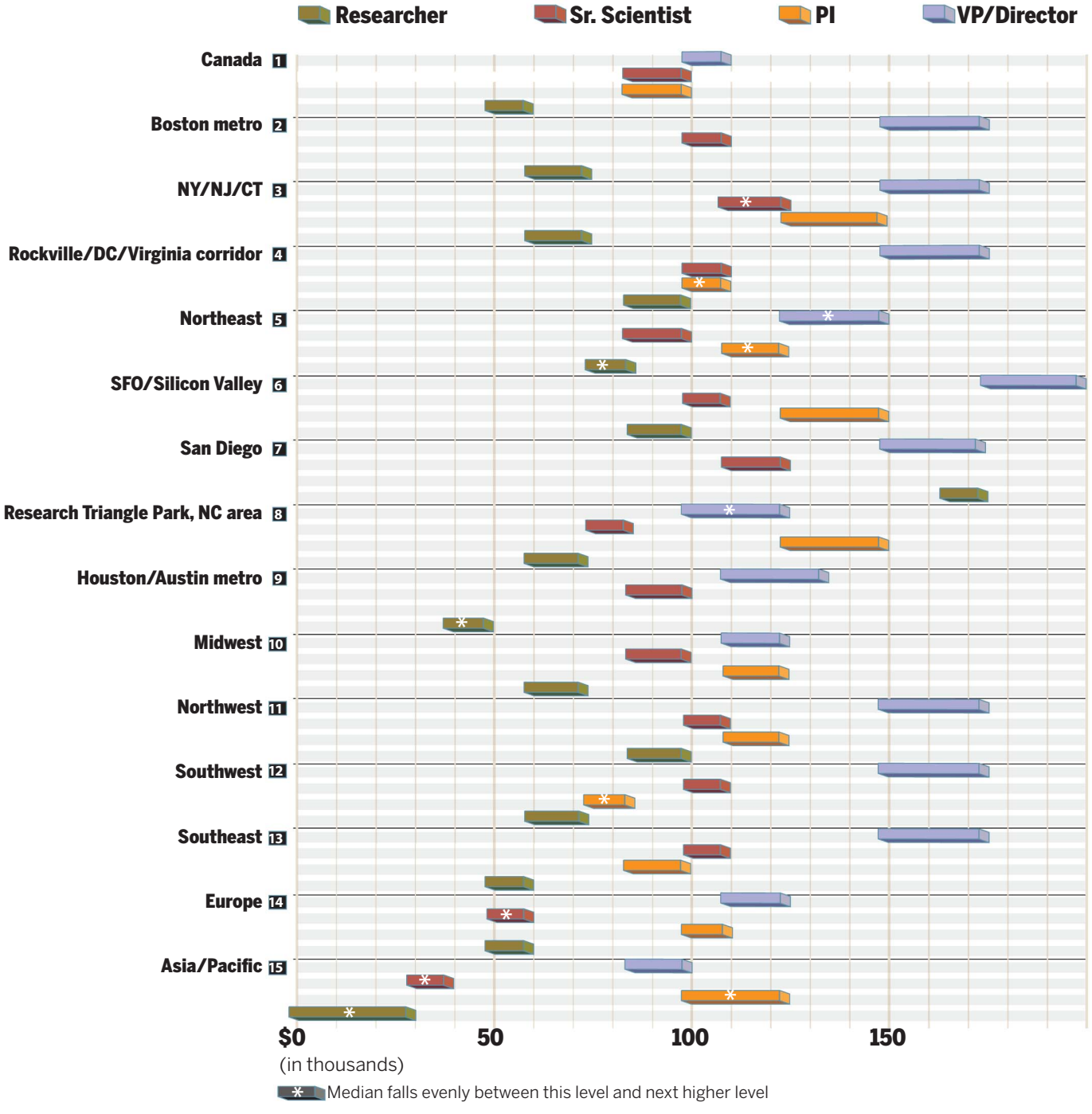
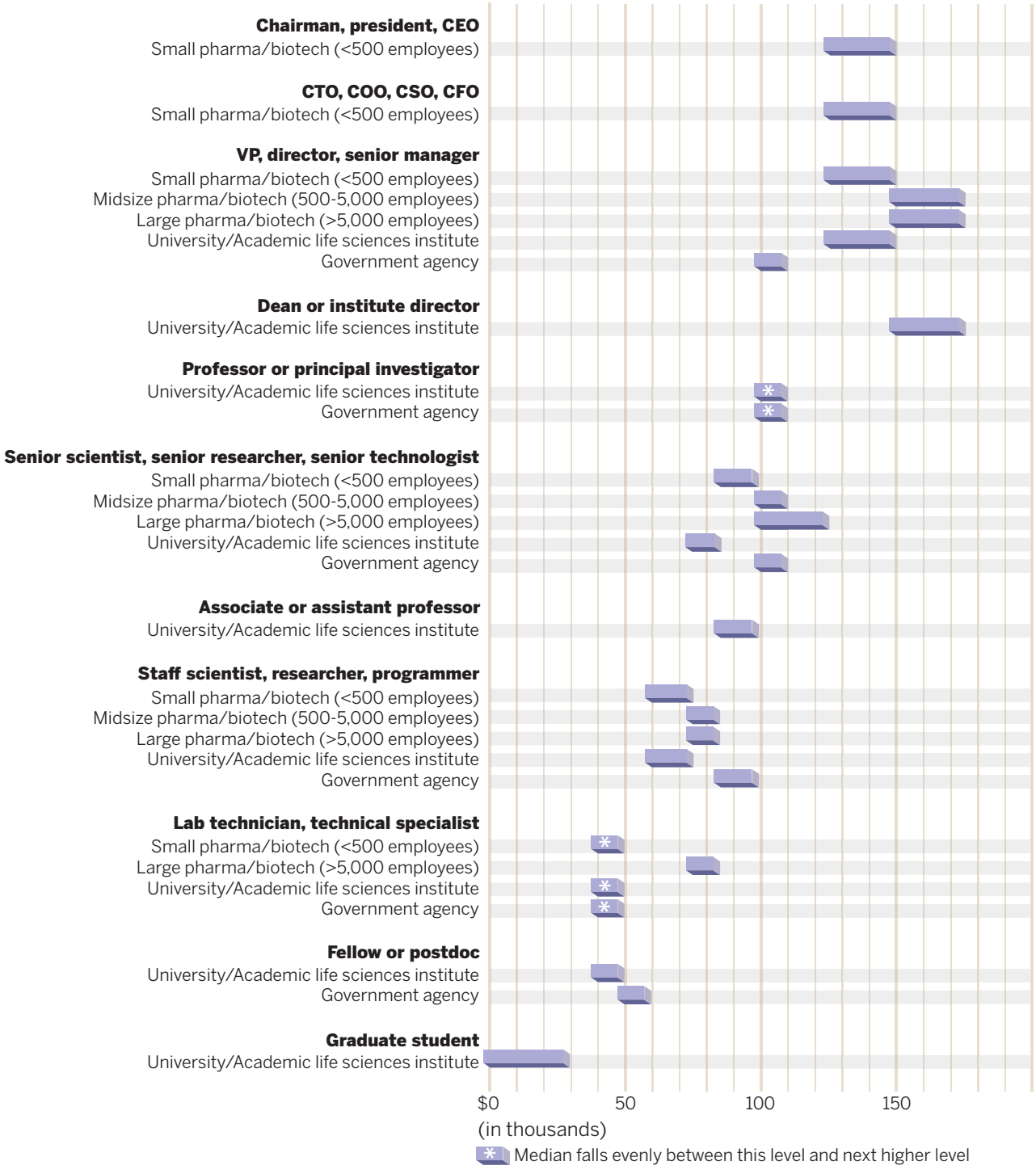
7
8
9
10
11
12
13
14
15


Researcher
Sr. Scientist
PI
VP/Director
Canada 1
Boston metro 2
NY/NJ/CT 3
Rockville/DC/Virginia corridor 4
Northeast 5
SFO/Silicon Valley 6
San Diego 7
Research Triangle Park, NC area 8
Houston/Austin metro 9
Midwest 10
Northwest 11
Southwest 12
Southeast 13
Europe 14
Asia/Pacific 15
$0
50
100
150
(in thousands)
Median falls evenly between this level and next higher level

Most Common Changes to Job in the Past Year

1. More responsibilities
2. More concerned about layoffs/pay cuts
3. Attending fewer conferences
4. Working longer hours

Median Salary by Title and Organization Type



What's on Your Mind

You weren't shy about sending in your career questions. *GT* talked to scientists who have been there and done that to get you some answers. *By Meredith W. Salisbury*

Job search

How do you interview for positions and ask for recommendations when you don't want your employer to know that you're looking for another job?

First, consider whether secrecy is really as important as your knee-jerk reaction says it is. "If you're in academia, the notion of a secret job search is sort of anathema [but] in industry it's pretty much the norm," Mark Borowsky says.

If you have a really good boss who would understand why you're feeling the need to look for another opportunity, David Barker says, it may be worth talking to him or her. That's one source of a recommendation.

And if you do decide to keep your job search off the radar, just "be direct with the people that you're contacting and tell them that this is confidential," Barker adds. In that case, you'll have to use references from prior jobs.

How do I handle gaps in employment?

Whatever you do, don't try to hide gaps. "Certainly when I interviewed people, the gaps just jump right out," Barker says. "I think you have to deal with them directly. … Everybody interviewing looks for these gaps and will ask about them anyway."

How do you give a job talk when your previous employers won't let you show your data?

Don't panic, Barker says. A job talk isn't necessarily about data that you generated the week before. "In a job talk, what people are looking for is as much presentation style [and] confidence," he says. It's normal for companies to keep their latest data confidential, but Barker says that industry scientists tend to forget that the rest of the community may not be aware of what's been going on at any given company during the past couple of years. He recommends using slightly older information in your job talk that's been made public, but would probably still be interesting to your audience. Specifically, he says, "talk about what your role was in it" and remember that "it's not so much what you talk about as how well you do it."

How do you negotiate salary for a new job?

Just like any other negotiation, you want to have all your facts, says Borowsky. Talk to your colleagues and mentors — particularly "people who are a little further along the career path than you" — and ask what appropriate pay for that kind of position would be. "Don't overlook the benefits," Borowsky adds. "The most important thing is to have a really solid and detailed understanding of what you're asking for and how it fits into the landscape."

When you're talking to people to find out what the right salary range is, says Tim Gardner, try to match the region and level of position so

Our panel of experts took on your toughest career questions:

DAVID BARKER, retired in 2006 as chief scientific officer of Illumina and now serves as an advisor to several startups

MARK BOROWSKY, director of bioinformatics at Massachusetts General Hospital

PAUL FLICEK, team leader for vertebrate genomics at the European Bioinformatics Institute

TIM GARDNER, associate director of computational biology at Amyris Biotechnologies

JOAN HERBERS, a biology professor at Ohio State University and president-elect of the Association for Women in Science

WIN HIDE, founder of South African National Bioinformatics Institute and visiting professor at Harvard School of Public Health

you get comparable data. Also, he notes, don't stress over it. Once an organization has made you an offer — even if it's not what you'd hoped for — you know the hiring team is interested in you. "Try to understand their perspective," Gardner says, and then go through what you truly need to make the offer work. "Be creative about how they can meet your needs," he says. "Most companies are willing to negotiate" but flexibility and understanding are critical for both parties.

When is the best time to look for a new job?

While "when you're sick of your current job" seems like the right answer, Gardner says that you should look to move when things are going well in your career. "You want to sell yourself when you're on top, not on bottom," he says.

Negotiations, transitions, and advancement

I've heard that there are salary differences between men and women who work at the same level. Why is this? Do most men negotiate for salary, even as postdocs?

"We know that women tend not to negotiate the same entry-level salaries as men, but they also don't get promoted at the same rate," Joan Herbers says. For example, a small study of engineering majors at Carnegie Mellon University showed that the starting salary negotiated by women graduates was about $4,000 less than that negotiated by their male counterparts.

"What the studies show us is that young women think that the playing field is absolutely level," Herbers adds; most women, she says, don't figure out the need for negotiation until their late 30s or early 40s. And while salary differences between men and women may not start out as that significant, even so-called "micro-inequities" add up to a major disparity over time, Herbers says. "Women need to understand the world of negotiation, and need to accept that this is part of the deal. Women give up sooner than men as well, even when they do negotiate."

Once you understand what's required for a promotion, keep track of everything you accomplish.

How do you ask for a promotion?

First, go to your boss and have a chat, says Win Hide. "Say that you're planning your career development" and that as part of your assessment, you'd like to know where you stand in terms of opportunities for promotion, he says. That's a good way to "establish what your boss regards as milestones for promotion" without being overly aggressive or demanding. Once you understand what's required for a promotion, keep track of everything you accomplish and sit down with your boss again once you feel you've earned it.

How do you deal with subtle sexism — including how to not let it hinder opportunities to advance?

Sexism in science isn't what it used to be, Herbers says. "The instances of blatant sexism have declined. … It's not as bad as when I was a grad student." However, she says, what remains is "the insidious stuff that nobody intends." One example: holding meetings outside normal work hours, which tends to affect women more as they're generally responsible for child care.

In general, Herbers advises people to compare notes with each other if a situation doesn't feel right or you think that something hasn't been handled properly. "Knowledge is key," she says. "Then you don't assume it's your fault." This is a good practice for scientists of all ages, she adds.

For senior researchers who have more than 20 years of experience but are not ready to retire for at least another 10 to 20 years, what is the best way to make a career change?

"At that point in your career, you can get rejuvenated by taking a risk, going to do something new," Barker says. That could be in the form of going to work for a startup, switching your research focus, or starting your own company, among other possibilities. "Taking a little risk and getting some changes … keeps people growing and learning new things," he adds.

How do you go from general programming to a more scientific focus?

While you could "learn some of the specialized biology packages, like Bioperl and BioJava," Borowsky says, your time may be better spent learning approaches to biological data, such as heavy-duty statistics packages. And be targeted about how you learn. "If you think you're going to go after a job in human genetics, then learn about genome-wide association studies," he adds.

Hide recommends getting a graduate degree, or taking on a project "that gives you an opportunity to

learn the biology of the system." He also suggests attending relevant workshops and meetings, which he says are "a very effective way of getting trained."

Working abroad

What's the best strategy when you want to work in a country with normally lower salaries than where you currently work?

Hide says he faced this problem frequently while running his bioinformatics institute in South Africa. "The hiring place often is not in a position to offer an internationally competitive salary," he says, "but they are in a position to let the scientists raise their own funds." Make sure the salary you're accepting is competitive locally, and get an agreement from the organization allowing you to compete for international grants. Hide says he "topped up" his institute salary with "grants from overseas," which gave him the level of compensation he was looking for.

Hide says he "topped up" his institute salary with "grants from overseas," which gave him the level of compensation he was looking for.

Paul Flicek says this is a question he hears a lot while recruiting as well. He tells people to look at the median salary of the country they currently live in and figure out how their salary relates to that; then, look at the median salary in the country you're thinking of moving to and see how the salary being offered compares. "That's a really good way to judge how you will live in that country compared to how you live [now]," Flicek says.

What's the best route to taking a postdoc in another country? Are there visa requirements?

Most countries offer some kind of training visa, says Flicek. "For the most part, the host institute is your first point of contact" for cutting through the red tape to get a position. ■

PROTEOMONITOR

Proteomics International IDs Venom for Drugs

Spiders, snakes, and centipedes may be the stuff nightmares are made of, but for one Australian firm they may form the foundation for its future.

In April, Proteomics International announced the development of Bioven, a process that links mass spectrometry with proprietary algorithms to determine peptide identity and predict their functions. Using this process, the company said that up to five times as many potential drug candidates can be recovered from venom than had been previously achieved.

In the first few months of operation, the company says, it was able to detect "several thousand" molecules and predict their potential bioactivity.

"We're extremely pleased with the number of molecules that we're identifying and the sheer number within each venom and in total," Proteomics International's managing director and cofounder Richard Lipscombe says. "And in terms of the analysis, we are in the process of evaluating leads that we've got from the process. We've been synthesizing a number of the peptides and they're currently undergoing validation."

While he declines to describe in detail the Bioven process, he says the company has been developing "methods at the front end" in order to look at proteomes more efficiently. The Bioven process is based on mapping the proteome of venoms using Proteomics International's proprietary algorithms to interpret the mass spectra and determine the protein sequence. New peptides and protein signatures are then analyzed against the company's in-house database of bioactive molecules to determine their applicability as potential therapeutic agents.

The method, Lipscombe says, has resulted in greater coverage of the venom proteomes that the company has been studying: While the scientific literature suggests about 50 to 100 peptides in any given venom, Proteomics International is seeing as many as 300 peptides, he says.

For now, the firm is targeting peptides that may have therapeutic use as antimicrobials and analgesics as proof of concept for its technology.

— *Tony Fong*

DATAPOINT

70

THE NUMBER OF WELL-CHARACTERIZED MONOCLONAL ANTIBODIES AGAINST 26 CANCER TARGETS IN THE DEVELOPMENTAL STUDIES HYBRIDOMA BANK AT THE UNIVERSITY OF IOWA

Proteomics Notes

The Irish **UCD CONWAY INSTITUTE**'s Proteome Research Centre is going to be a reference center for the Swedish firm **DENATOR**'s Stabilizor T1 system for stabilizing tissue samples.

OVERBROOK SCIENTIFIC will distribute Phytronix's laser diode thermal desorption ionization technology in the US. According to Overbrook, the LDTD technology can increase the speed of analyzing compounds 20 to 100 times as compared to LC/MS-based techniques.

The **HUMAN PROTEOME ORGANIZATION** is now accepting nominations for its board of directors. Nominees must be in good standing with HUPO, and the nominations must be made and seconded by a HUPO member. Elections will be held during the annual conference in September.

FUNDED GRANTS

$186,969/FY 2009

PROTEOMIC STUDIES OF DENDRIMER-BASED NANOMEDICINES

Grantee: Weiguo Andy Tao, Purdue University
Began: Mar. 1, 2009; Ends: Feb. 29, 2012

Tao plans to develop a proteomic approach to study nanomedicine based on dendrimer-protein tyrosine phosphatase inhibitor conjugates. According to the grant abstract, he will synthesize dendrimers that contain immobilized PTP inhibitors. Then Tao will identify PTP targets in cancer cell homogenates and protein targets in intact cancer cells in culture.

$299,796/FY 2009

PROVIDING PEPTIDE ATLAS BASED SERVICES THROUGH THE CAGRID INFRASTRUCTURE

Grantee: John Boyle, Institute for Systems Biology
Began: Jan. 1, 2009; Ends: Jun. 30, 2011

With this grant, Boyle will be able to make PeptideAtlas available to researchers and clinicians. To do that, PeptideAtlas needs to be supported by a framework that supports and can portray rich semantics, has a high level of interoperability, and is a distributed system so that information is readily available at all times. To accomplish that, Boyle plans to implement caGRID.

INSEQUENCE

Updated Polonator Is Ready for Rollout; Price: $170K

About a year after first presenting the Polonator sequencer to the scientific community and shipping instruments to early-access users, Dover, a Danaher Motion company, is now ready to sell an upgraded version of the instrument to a wide range of customers.

This spring, the company, which has been co-developing the low-cost, nonproprietary, high-throughput sequencing platform with George Church's group at Harvard Medical School, shipped an instrument to the University of Utah, its fifth customer to date and the first lab outside the early-access circle of users.

Over the next year or so, the developers plan to make additional improvements, including reagent kits, increased read lengths, and a simpler library preparation protocol. By 2010, they expect the output per run to increase 10-fold, to 100 gigabases.

The recent upgrades, which include changes to the flow cell, fluidics, operating software, and instrument cover, resulted from feedback from early-access customers, according to Kevin McCarthy, chief technology officer of Dover. "It took longer than we expected to address the issues raised in our early-adopter phase, but all changes have been implemented, and we now have a robust, high-performance sequencer," he says.

Without bead enrichment, the instrument currently generates more than 10 million mappable reads per flow cell lane, or about 5 gigabases of data per run, based on 2x13-base gapped paired-end reads and two 8-lane flow cells. Bead enrichment is expected to double the output per run to 10 gigabases, according to McCarthy.

Consumables costs for generating a megabase of data, or 40,000 sequence tags, are currently $1, and are expected to drop approximately 10-fold over the next year, he says. The instrument itself now costs $170,000, which is $20,000 more than a year ago, due to price increases for a number of components, including the camera. Setup costs are not included in this price.

— *Julia Karow*

DATAPOINT

$17

MILLION

AMOUNT OF NEW GRANT FUNDING FROM THE JAPANESE GOVERNMENT THAT WILL ALLOW THE RIKEN OMICS SCIENCE CENTER TO EXPAND ITS DNA SEQUENCING INFRASTRUCTURE.

Sequencing Notes

THE NATIONAL CANCER INSTITUTE plans to expand two cancer genome sequencing projects thanks to stimulus funding. The Cancer Genome Atlas project is expected to now include 20 to 25 tumor types, and the institute will apply next-generation sequencing to at least 100 tumor samples for its TARGET initiative.

LIFE TECHNOLOGIES reported "high double-digit growth" in first-quarter revenue from SOLiD sequencing systems along with increased revenues from capillary electrophoresis instruments and consumables. Meantime, **ILLUMINA** said it shipped "a record number" of sequencers during the first quarter, noting that the Broad Institute acquired 22 additional GAs, while the **BEIJING GENOMICS** Institute purchased 12 additional instruments.

FUNDED GRANTS

$430,880/FY 2008

DESIGNS AND METHODS FOR SEQUENCE-BASED VALIDATION ANALYSIS

Grantee: Lue Ping Zhao, Fred Hutchinson Cancer Research Center

Began: Sep. 25, 2008; Ends: Jun. 30, 2011

According to the abstract, the short-term goal of this project "is to develop novel statistical designs that enable researchers to design cost-effective study designs to validate GWAS discoveries using resequencing technologies" and "to develop statistical methods for assessing DNA sequence data features."

$462,728/FY 2008

STATISTICAL METHODS FOR THE DESIGN AND INTERPRETATION OF DEEP RESEQUENCING STUDIES

Grantee: Shamil Sunyaev, Brigham and Women's Hospital

Began: Sep. 30, 2008; Ends: Jun. 30, 2011

The National Institute of Mental Health awarded this grant to Sunyaev to develop new statistical methods for targeted and genome-wide sequencing approaches, including "identifying causal variants inside a targeted region, such as a GWAS peak or candidate gene" and "to optimally capture the association signal."

Genome-Wide RNAi screening - Now at your benchtop

Decode™ RNAi viral screening pools combine the advantages of microRNA-adapted shRNA (shRNAmir) design, the power of viral delivery and the convenience of ready-to-use pooled viral particles to facilitate genome-wide multiplexed RNAi screens without the high cost and labor associated with individually arrayed screens.

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- **Discovery Potential** - Targets annotated and unknown genes
- **Efficient low copy knockdown** - Proven with GIPZ lentiviral shRNAmir
- **Lentiviral Delivery** - RNAi screens are now possible in primary, non-dividing and hard-to-transfect cells
- **Visual Tracking** - TurboGFP marks shRNAmir expressing cells
- **Easily identify hits** - with barcode microarrays or PCR sequencing

NEW! Negative selection screening kits with barcode microarrays.

"As beta users of the Open Biosystems' Decode RNAi viral pools, we are very happy with its convenient format, knockdown performance in multiplexed RNAi screens, as well as, its ability to transduce many different cell lines. Researchers at the Peter Mac are very excited about the possibilities of being able to comb through thousands of genes at a time to further our understanding of cancer biology and ultimately the search for novel cancer therapeutics."

- Ricky Johnstone, PhD Group Leader, Gene Regulation
- Peter MacCallum Cancer Center, Melbourne, Australia

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RNAiNEWS

RNAi Shops Focus on Developing Tech in House

With delivery remaining the key problem facing the successful development of RNAi therapeutics, an increasing number of players in the field are looking within their own labs for solutions, often using delivery technologies as the foundation upon which to build their businesses.

Although the need for effective delivery approaches has long been recognized, many of the early entrants into the RNAi therapeutics space — companies such as Alnylam Pharmaceuticals, Sirna Therapeutics (now a part of Merck), Atugen (now Silence Therapeutics), and CytRx (which spun out its RNAi drug operations into RXi Pharmaceuticals) — focused much of their initial research and development on the molecules themselves.

But with a greater understanding of how to design and modify functional siRNAs and licenses to key intellectual property now widely available, many RNAi drug firms are making delivery their first priority.

Topping the list is Tekmira Pharmaceuticals, which secured access to one of the RNAi drug field's most important delivery technologies when it merged with Protiva Biotherapeutics about a year ago.

That technology, termed SNALPs, or stable nucleic acid lipid particles, was developed by Protiva and comprises nucleic acids encapsulated by cationic and fusogenic lipids surrounded by a polyethylene glycol coating to prevent the positively charged cationic lipid from clearing the bloodstream.

The technology proved so promising that both Alnylam and Sirna took early licenses, although a lawsuit between Protiva and Tekmira predecessor Inex Pharmaceuticals ultimately led to a restructuring of the arrangement with Sirna. Alnylam, however, continues to use the technology.

The litigation was settled through the Tekmira/Protiva merger, and Tekmira has since attracted a number of additional companies interested in using SNALPs in their own RNAi efforts, including Roche, Johnson & Johnson, and Bristol-Myers Squibb.

With revenues from these and other collaborations, Tekmira has been able to fund its own siRNA drug pipeline.

—Doug Macron

DATAPOINT

$1.2

MILLION

AMOUNT NOVARTIS PAID FOR 65,922 UNREGISTERED SHARES OF ALNYLAM PHARMACEUTICALS.

RNAi Notes

ALNYLAM PHARMACEUTICALS and **ISIS PHARMACEUTICALS** have formed a collaboration to develop Isis' single-stranded RNAi technology. Alnylam received a co-exclusive license to Isis' ssRNAi technology, in exchange for which Isis will receive upfront payments, research and development milestones, and royalties.

ROSETTA GENOMICS closed a deal with **PROMETHEUS LABORATORIES** that gives Prometheus the US market rights to Rosetta's three microRNA-based diagnostics: miRview Meso, miRview Mets, and miRview Squamous.

The **US FOOD AND DRUG ADMINISTRATION** approved **TEKMIRA PHARMACEUTICALS**' investigational new drug application for ApoB SNALP, a siRNA-based hypercholesterolemia drug that targets apolipoprotein B.

FUNDED GRANTS

$282,600/FY 2009

DOUBLE-STRANDED RNA-MEDIATED SIGNALING PATHWAY AND GENE SILENCING

Yi Liu, University of Texas Southwest Medical Center
Began: Apr. 1, 2009; Ends: Mar. 31, 2011

For this grant Yi Liu will be studying the signaling pathway responsible for dsRNA-induced gene transcription in the filamentous fungus *Neurospora crassa* through forward and reverse genetics. They will also investigate the biogenesis and function of DNA damage-induced small RNAs and the mechanism of aberrant RNA production after that damage.

$210,574/FY 2009

ROLE OF RNA SILENCING IN TELOMERE DYNAMICS

Zhixin Xie, Texas Tech University
Began: May 1, 2009; Ends: Apr. 30, 2012

Zhixin Xie plans to use both genetic and biochemical approaches to elucidate the role of RNA silencing in telomere dynamics. He will investigate the origin and mechanism of tel-siRNAs and determine tel-siRNAs' part in telomere dynamics by using the tel-siRNA deficient mutants. He also plans to characterize the the tel-siRNA-associated effector complex.

Upstream | MICROARRAYS

Microarray Notes

GOLDEN HELIX has modified its SNP & Variation Suite software to provide tools for **AGILENT**'s copy number variation microarray data, including association studies, data prep, quality assurance, interaction analyses, study review, and predictive modeling.

Scientists at **DUKE UNIVERSITY**'s Institute for Genome Sciences and Policy will use **WAFERGEN**'s SmartChip Real-Time PCR system to validate polymorphisms associated with breast cancer prognosis and response to therapy.

A team led by **MEMORIAL SLOAN-KETTERING CANCER CENTER** used gene expression analysis to find three genes involved in breast cancer metastasis to the brain: COX2, an epidermal growth factor receptor ligand gene, and α2,6-sialtransferase ST6GALNAC5.

BIOARRAYNEWS

Illumina Lawsuit: Affy's GeneTitan Infringes Patents

Last month, Illumina filed a lawsuit against Affymetrix that alleges that the company's new GeneTitan automated platform, as well as several of the system's components and related products, infringe Illumina's array technology.

The new litigation commenced nearly a year and a half after Illumina paid Affy a one-time $90 million payment to settle multiple suits that Affy had filed in the US, Germany, and UK between 2004 and 2007. Illumina did not admit liability as part of the settlement.

The new suit, filed May 4 in the US District Court for the Western District of Wisconsin, alleges that a variety of Affymetrix products infringe Illumina's US Patent No. 7,510,841, entitled, "Methods of Making and Using Composite Arrays for the Detection of a Plurality of Target Analytes." The US Patent and Trademark Office awarded the patent to Illumina on March 31.

The Affy products named in the suit include the GeneChip HT RG-230 PM Array Plate, the GeneChip HT Array Plate Scanner, the GeneChip HT 3' IVT Express Kit, the GeneChip Array Station, and the GeneTitan instrument.

In a research note published by Leerink Swann analyst Isaac Ro, he described the new suit as "Round 2" of litigation between the companies. Ro predicted that it would have "no material impact" on Illumina, which he described as "actively seeking to protect its IP," and described it as "marginally negative" for Affy.

"We think this news could pressure [Affy's] existing burn rate and note that the new line of peg arrays [named in the suit] is key to reducing [Affy's] manufacturing costs and cost per data point," Ro said.

Affy launched the products named in the suit last September as part of the rollout of its new upgraded microarray platform. The system, priced at roughly $300,000, includes Affy's ArrayStation, launched in 2005, which fulfills automated sample-preparation and liquid-handling duties, while the GeneTitan provides all other array-processing steps.

— *Justin Petrone*

DATAPOINT

5

NUMBER OF COMPANIES THAT ANNOUNCED THE ABILITY OF THEIR RESPECTIVE FLU CHIPS TO DETECT INFLUENZA A, SUBTYPE H1N1, ALSO KNOWN AS THE SWINE FLU.

FUNDED GRANTS

$441,239/FY 2009

ENTHALPY ARRAY SCREENING AND RANKING OF DRUG FRAGMENTS FOR DRUG DISCOVERY

Grantee: Francisco Torres, Palo Alto Research Center
Began: May 1, 2009; Ends: Apr. 30, 2012

Torres will develop enthalpy array technology for pre-screening fragments prior to full functional probe screening in order to increase drug target screening efficiency. In addition, enthalpy arrays can be used to determine binding strength, specificity, and binding enthalpy for use in fragment elaboration, the step after fragment hits are established, the abstract states.

$199,260/FY 2009

A NOVEL ARRAY FOR DETECTION OF UNSTABLE TANDEM REPEATS

Grantee: Russell Margolis, Johns Hopkins University
Began: Mar. 14, 2008; Ends: Feb. 28, 2010

In collaboration with Evan Eichler and NimbleGen, Margolis will develop an oligonucleotide array specifically designed to detect changes in the number of repeating units in over 3,000 tandem repeats. He will test the array on 80 people with schizophrenia, who may harbor significant disease-causing variations of longer tandem repeats, genes not covered by conventional CNV arrays.

BIOINFORM

Microsoft Offers Updates from BioIT Alliance

At the recent the Bio-IT World Conference & Expo, Rudy Potenzone, Microsoft's industry technology strategist for pharmaceuticals, presented a panel of diverse collaborations underway through the BioIT Alliance, an organization Microsoft formed in 2006 to link vendors in the life sciences space to "explore new ways" of data sharing and to better leverage IT to foster personalized medicine.

One of the new collaborations is with the Pistoia Alliance, a cross-pharma collaborative venture that seeks to "streamline noncompetitive elements" of drug-discovery workflow. Named after the Tuscany town in which the first meeting took place last year, it was founded by discovery informatics researchers from AstraZeneca, GlaxoSmithKline, Novartis, and Pfizer, and launched publicly in February.

Another project is in the realm of chemistry and authoring. A division called Microsoft External Research that supports projects with firms, industry, and governments, has supported the development of chemistry-related authoring and rendering in Word 2007 documents through a partnership with chemist Peter Murray-Rust from Cambridge University's Unilever Centre for Molecular Science Informatics.

At the BioIT Alliance session, Murray-Rust demonstrated Chem4Word for the first time in public and said it will be released in the early summer. Murray-Rust, an open-source and open-data advocate, created Chemical Markup Language, CML, which is an XML-language for chemical information used for representing molecules, spectra, reactions, and computational chemistry.

Another venture in the BioIT Alliance portfolio is an open source life science Web registry project called BioCatalogue, described in *Nature Proceedings*, spearheaded by Carol Goble and colleagues from the University of Manchester's School of Computer Science and colleagues at the EMBL European Bioinformatics Institute.

It began as a collaboration between EMBL-EBI and the myGrid project at the University of Manchester.

— *Vivien Marx*

DATAPOINT

100

THOUSAND

THE NUMBER OF VOLUNTEER GENOME SEQUENCES THAT WILL BE STORED ON ISILON HARDWARE PURCHASED BY THE PERSONAL GENOME PROJECT

Bioinformatics Notes

INFORSENSE says that **CELERA** is using the firm's platform to integrate internal and public data to identify biomarkers. InforSense's Translational Research Solution will allow Celera scientists to browse enzyme-linked immunoassay, SNP, and other data.

COMPENDIA BIOSCIENCE is partnering with **MDS PHARMA SERVICES** to create OncoPredictor, a platform to improve cancer drug development. The platform combines MDS's OncoPanel with Compendia's Oncomine so drug developers can see which patients are likely to respond to a new therapy.

Data mining firm **DECISIONQ** and **THOMAS JEFFERSON UNIVERSITY'S** Kimmel Cancer Center are using predictive analytic and machine learning to mine the university's cancer registries and tailor patient treatment.

FUNDED GRANTS

$311,442/FY 2009

CONTINUAL DEVELOPMENT OF PROTEIN DOCKING ALGORITHMS

Grantee: Zhiping Weng, University of Massachusetts Medical School, Worcester

Began: May 1, 2009; Ends: Apr. 30, 2013

This funding will be applied to the development of computational methods, algorithms, and software to facilitate three-dimensional protein-protein structure predication. Weng and his lab have recently developed a new energy function, IFACE, which improves the performance of ZDOCK, a protein docking algorithm.

$407,935/FY 2009

BIOINFORMATICS TOOLS FOR THE ANALYSIS OF THE SPATIOTEMPORAL ORGANIZATION OF PROTEIN EXPRESSION IN NEURAL FUNCTIONAL UNITS

Grantee: Eduardo Macagno, University of California, San Diego

Began: Apr. 15, 2009; Ends: Mar. 31, 2010

This grant will go toward funding the development of computational tools to support the study of the development and repair of the nervous system at the molecular level. The researchers plan to design new tools to analyze data obtained via mass spectrometry imaging.

PGx & Molecular Dx Notes

The **US CENTERS FOR MEDICARE & MEDICAID SERVICES** found insufficient evidence to demonstrate the effectiveness of pharmacogenomics-guided warfarin dosing in improving health outcomes and deemed the use of pharmacogenomic testing for this to be unnecessary.

IPSOGEN, a French molecular diagnostics company, licensed non-exclusive rights to use gene variants for diagnosing leukemia to the University of Utah's **ARUP LABORATORIES**.

BECTON DICKINSON and **FUJIREBIO DIAGNOSTICS** signed a worldwide development and supply agreement for multiplex oncology diagnostic assays. The companies will develop diagnostic products that contain Fujirebio's biomarkers that will run on BD's multiplex testing platform.

PHARMACOGENOMICSREPORTER

Clinical Data Inks PGx Deal with U of Pittsburgh

Clinical Data has entered into a collaboration with the University of Pittsburgh to discover and validate Fc gamma receptor gene markers that may eventually lead to the development of new pharmacogenetic tests that gauge response to monoclonal antibodies, such as Herceptin, Rituxan, and Erbitux.

The research partnership, announced last month, will expand Clinical Data's existing FCGR program under its PGx Health division, which includes the PGxPredict: Rituximab test and research collaborations with other institutions.

"Our goal is to validate the biomarkers or genetic variants associated with response and potentially discover other genetic variants in this gene and their impact on response to IgG1 mAb-based therapies," according to a Clinical Data spokesperson. The company aims "to utilize this information in developing tests to predict drug response, as we have done with our PGxPredict: Rituximab test."

The announcement is in line with the company's previously outlined 2009 strategic goals, which include driving adoption of its Familion and PGxPredict brand of assays in the cardiovascular disease market, and establishing research collaborations to discover and validate genetic variants in the FCGR pathway that predict response for monoclonal antibodies.

Under the research collaboration between the University of Pittsburgh and Clinical Data, the partners are planning to conduct a series of clinical trials to gauge the link between FCGR gene variants and response to mAb-based therapies, such as Erbitux (cetuximab), Rituxan (rituximab), Herceptin (trastuzumab), and potentially other mAb cancer drugs in the IgG1 subclass.

The initial research program between PGxHealth and the university will focus on gauging responsiveness to Erbitux in head and neck cancer patients. Robert Ferris, associate professor and chief of the head and neck surgery division at the University of Pittsburgh Cancer Institute, will lead the study.

— *Turna Ray*

DATAPOINT

$1

MILLION

NCI AND THE CANARY FOUNDATION HAVE TEAMED UP TO OFFER $1 MILLION GRANTS TO STUDIES OF GENETICS AND DIAGNOSTICS FOR LUNG CANCER.

FUNDED GRANTS

$66,400/FY 2009

HEREDITARY BREAST CANCER AND NOVEL HISPANIC BRCA MUTATIONS

Grantee: Jeffrey Weitzel, City of Hope
Began: Mar. 1, 2009; Ends: Feb. 28, 2011

Weitzel and his colleagues will use the funds to study a panel of BRCA mutations previously linked to women of Hispanic ancestry to pre-screen samples to determine whether this ancestry demonstrates clinical utility. The team will collect DNA samples and clinical data from Hispanic patients and then "use ancestral informative markers to characterize the admixture of carriers."

$146,568/FY 2009

MICROMAGNETIC APTAMER PCR SYSTEM FOR ULTRASENSITIVE MULTIPLEXED PROTEIN DETECTION

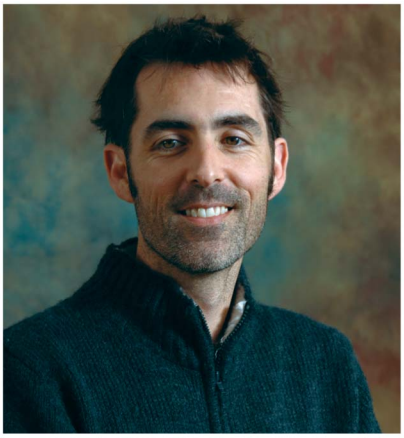
Grantee: Hyongsok Tom Soh, University of Califonia, Santa Barbara
Began: May 1, 2009; Ends: Apr. 30, 2011

Soh proposes to develop a "Micro-Magnetic Separation — Quantitative Polymerase Chain Reaction" system integrating chip-based micro-magnetic separation with aptamer-based quantitative PCR to help alleviate the problem of detecting and quantifying protein biomarkers of low abundance in clinical samples.

'An Artificial Argument'

In discussing the effects of genetic variants on disease, researchers have divided into two camps: the common disease/common variant side and the rare variant side. What if they are both wrong, and both right?

Recently, researchers have been discussing how genetic variants may affect a person's disease risk. Andrew Singleton, a researcher in the National Institute on Aging's neurogenetics lab, tells GT's Ciara Curtin that both common and rare variants — as well as those that fall in between — may play a role in disease.



ANDREW SINGLETON

GENOME TECHNOLOGY: What do you think of the common disease/common variant hypothesis? Has it been helpful in understanding disease risk?

ANDREW SINGLETON: You have groups taking total opposite views and almost creating an artificial argument. The artificial argument at the moment is common disease/common variant hypothesis or is disease modulated by rare risk variants. They are not mutually exclusive hypotheses. Clearly, the results of genome-wide associations have identified lots of risk variants for common diseases and these are, by definition, common risk variants. There's without doubt merit to the common disease/common variant hypothesis. I think that what we're starting to get a handle on is that there aren't many things like ApoE, for Alzheimer's disease, where it is a common variant and it exerts a really strong effect. For really common diseases, what we're seeing is common risk alleles that exert really small effects [on] lifetime risk for disease and there might be many of these for common disease. Now, that doesn't preclude there being rare variants that affect disease also.

GT: What do you think the role of rare variants will be?

AS: I think they will be important in disease. The issue with rare variants is that they are going to be extremely hard to show that they are associated with disease. What we're seeing now is almost a gradient of effect. You have these rare disease-causing mutations which are often coding [and] almost invariably cause disease or really, really increase your risk for disease. Then we have coding variants that are common, but don't cause disease, but increase risk for disease. And then we have noncoding common variants which sneak up your risk for disease a little bit. I suspect continuing along there will be rare variants that are noncoding [but] that change lifetime risk for disease. I don't doubt that rare risk variants will exist for disease, but it will be really hard to prove what's the risk variant and what's just benign.

GT: What do you think is the best approach to go from the loci identified by GWAS to actual genes?

AS: I think the most accessible way is to look at the effects of risk variants on, for instance, expression or DNA methylation. One would presume, in the absence of a really striking coding variant on your risk haplotype, that the risk haplotype or the risk variant is affecting gene expression or gene splicing or DNA methylation or all of those things. I actually think that the way to do this is to create fairly large central resources where we measure common genetic variability across the genome and look at the expression in a genome-wide manner, DNA methylation in a genome-wide manner. This is the idea of the GTex project: This is a project that aims to assay common genetic variability in roughly 1,000 individuals and look at expression in 50 tissues from those individuals and create an atlas of effects of common genetic variability on expression.

GT: Anything you'd like to add?

AS: One thing that's often raised is what is the use of finding rare risk variants? What does it tell us about disease? For every locus that we find, and for every gene that we find associated with that locus, [it] gives us a bit more information about the underlying biology of the disease. ■

Antibody Array in Disguise

A Caltech lab's microarray aims to measure proteins in plasma rapidly and cheaply to help elucidate a patient's response to therapy. *By Jeanene Swanson*

Developing microfluidic devices might be tricky, but finding antibodies that work as all-around great detection agents is even trickier. Just ask Jim Heath, a chemist at Caltech whose aim is to combine recent advances in microfluidics with a novel strategy to create highly sensitive and specific antibodies for better detection of diagnostic and prognostic disease biomarkers. A big advance out of his lab is an integrated blood barcode chip, which can take a finger-prick's worth of blood and detect proteins in the plasma within five minutes. The chip consists of a DNA array which, when the time comes to use it, is transformed into an antibody array by flowing oligo-bound antibodies over it. The oligos bind the DNA, and the antibodies then serve as capture agents for the proteins in the blood.

"We'd like to be able to monitor a patient's response to therapy by looking at how certain proteins in their blood are dynamically evolving during the few-hour window that surrounds when they first receive the therapy," Heath says.

The biggest protein measurement problem is the protein capture agent.

When it comes down to it, antibodies are still the thorn in clinical detection's side. The biggest protein measurement problem is the protein capture agent, says Heath, and in his lab he's tried many alternatives, including aptamers, small molecule inhibitors, and the like. None of these could provide the high-affinity, high-stability alternative to antibodies, so Heath created his own method.

The method, called One Bead One Compound, is based on the concept of using "really big libraries of artificial and nonnatural amino-acid containing peptides and letting the protein assemble its own capture agent from those libraries," Heath says. "The protein serves as a catalyst to couple and build a covalent linkage between multiple peptides."

As an example, he says, consider mixing millions of beads in 20 different pots, each containing a unique amino acid. After mixing the beads together over multiple rounds, every bead is guaranteed to have a single, unique peptide on it. If you start with 100 million beads and 20 amino acids, says Heath, you can create a library of all possible variations of a 6-mer peptide.

The next step is to take this library, mix it with a target protein, and identify a peptide that binds to the protein but not very well. "It has lousy affinity and lousy selectivity," Heath says, "but it does bind." Then, scale that peptide up, add an azide-containing amino acid, and modify the ends of the rest of the peptide library with acetyl groups. If the protein, azide-bound peptide, and peptide library are all mixed together, the protein will search for peptides that best couple with the azide-peptide, already bound to it.

"So normally, you can get the azide and acetyl groups to react with a catalyst, but in this case the protein itself is the catalyst," Heath says. "And it only catalyzes the reaction when the two peptides are organized on its surface in just the right way — it'd be almost impossible to get it just right unless you've got a really big library."

It turns out that the bi- and tri-ligand peptides that the protein helps create are really selective. While a bi-ligand is already "pretty selective," being able to pull proteins out of blood, a tri-ligand is "not only very selective, it has antibody-like affinity," Heath says.

Heath has begun commercializing his capture agents and has set up a lab in Singapore to build out methods to make the process high-throughput. "Our overall goal is to do diagnostic and health measurements [of proteins] so they cost about a penny per protein you measure," he says, adding that right now they cost about $50. ■

Events | MEETINGS AND DEADLINES

Conferences

DATE	CONFERENCE	ORGANIZER	LOCATION	CATEGORY
Jun 1-5	Microbial Genomics & Metagenomics Workshop	Joint Genome Institute	Walnut Creek, Calif.	Genomics
Jun 6-11	Pathways, Networks and Systems Medicine Conference	Aegean Conferences	Corfu, Greece	Systems biology
Jun 8-10	Beyond Genome	CHI	San Francisco	Genomics
Jun 22-26	DIA Annual Meeting	Drug Information Assoc.	Boston	Pharma
Jun 27-Jul 2	International Conference on Intelligent Systems for Molecular Biology	ISCB	Stockholm	Bioinformatics
Jul 17-19	Genetic Alliance Annual Meeting	Genetic Alliance	Bethesda, Md.	Genetics
Jul 19-23	AACC Annual Meeting and Clinical Lab Expo	American Association for Clinical Chemistry	Chicago	Clinical
Jul 19-24	Human Genetics & Genomics	Gordon Research	Biddeford, Maine	Genomics
Jul 20-22	caBIG Annual Meeting	NCI Conference	Washington, DC	Cancer
Jul 25-29	Symposium of the Protein Society	Protein Society	Boston	Proteomics
Aug 3-6	Drug Discovery & Development of Innovative Therapeutics	IBC	Boston	Pharma
Aug 6-7	Microarray World Congress	Select Biosciences	South San Francisco, Calif.	Arrays
Aug 16-20	238th National Meeting of the American Chemical Society	ACS	Washington, DC	Chemistry
Aug 23-27	International Symposium on Mass Spectrometry in the Health and Life Sciences	UCSF	San Francisco	Proteomics
Aug 25-28	International Conference on Genomics: Human and Beyond	Beijing Genomics Institute	Shenzhen & Hong Kong	Genomics
Aug 30-Sep 4	10th International Conference on Systems Biology	ICSB	Stanford, Calif.	General
Sep 11-13	Human Genome Variation and Complex Genome Analysis		Tallinn, Estonia	Genomics
Sep 14-17	Personal Genomes	Cold Spring Harbor Laboratory	Cold Spring Harbor, NY	Personal genomics
Sep 16-18	5th International DNA Sampling Conference: The Age of Personalized Genomics	Genome Alberta	Banff, Alberta	Personal genomics
Sep 17-20	Microbial Genomes	Wellcome Trust	Hinxton, UK	Genomics
Sep 21-23	Exploring Next-Generation Sequencing	CHI	Providence, RI	Sequencing
Sep 23-25	Eukaryotic Annotation and Analysis Course	J. Craig Venter Institute	Rockville, Md.	Bioinformatics
Sep 23-27	Genomics of Common Diseases	Wellcome Trust	Hinxton, UK	Genomics
Sep 26-30	HUPO 8th Annual World Congress	HUPO	Toronto	Proteomics
Oct 4-7	AIRI Annual Meeting	Association of Independent Research Institutes	Seattle	General
Oct 5-7	Clinical Proteomic Technologies for Cancer Annual Meeting	NCI	Bethesda, Md.	Proteomics
Oct 17-21	Neuroscience 2009	Society for Neuroscience	Chicago	Neuroscience
Oct 20-24	ASHG Annual Meeting	American Society of Human Genetics	Honolulu	Genetics
Nov 2-4	Discovery on Target	CHI	Boston	Pharma
Nov 9-10	Burrill Personalized Medicine Meeting	Burrill & Company	San Francisco	Personalized medicine
Nov 9-11	Northeast Regional Life Sciences Core Directors Meeting	Cornell University	Ithaca, NY	Core labs

Deadlines

JUNE 2 Abstract submission deadline for the **ASHG** meeting, to be held from Oct. 20-24.

JUNE 2 Abstract submission deadline for the **AMP** meeting, to be held from Nov 19-22.

JUNE 3 Application deadline for the grant entitled **PHARMACOGENOMICS KNOWLEDGE BASE** issued by the National Institute of General Medical Sciences. The award will fund applicants to develop the PharmGKB, to house complete, comprehensive, and current knowledge in pharmacogenomics.

JUNE 3 Application deadline for the grant entitled **PHARMACOGENOMICS RESEARCH NETWORK**. Grantees will conduct research into understanding the genetic basis of variable drug responses, both therapeutic and adverse.

JUNE 15 Application deadline for the NSF grant, **TERAGRID PHASE III: EXTREME DIGITAL SOURCES FOR SCIENCE AND ENGINEERING** This will fund further infrastructure support for the TeraGrid, a computing resource for handling scientific and engineering digital information.

JUNE 19 Application deadline for **THE INTEGRATIVE CANCER BIOLOGY PROGRAM (ICBP): CENTERS FOR CANCER SYSTEMS BIOLOGY (CCSB)** grant. NIH will fund CCSBs to develop integrative systems approaches and mathematical/computational modeling for cancer research.

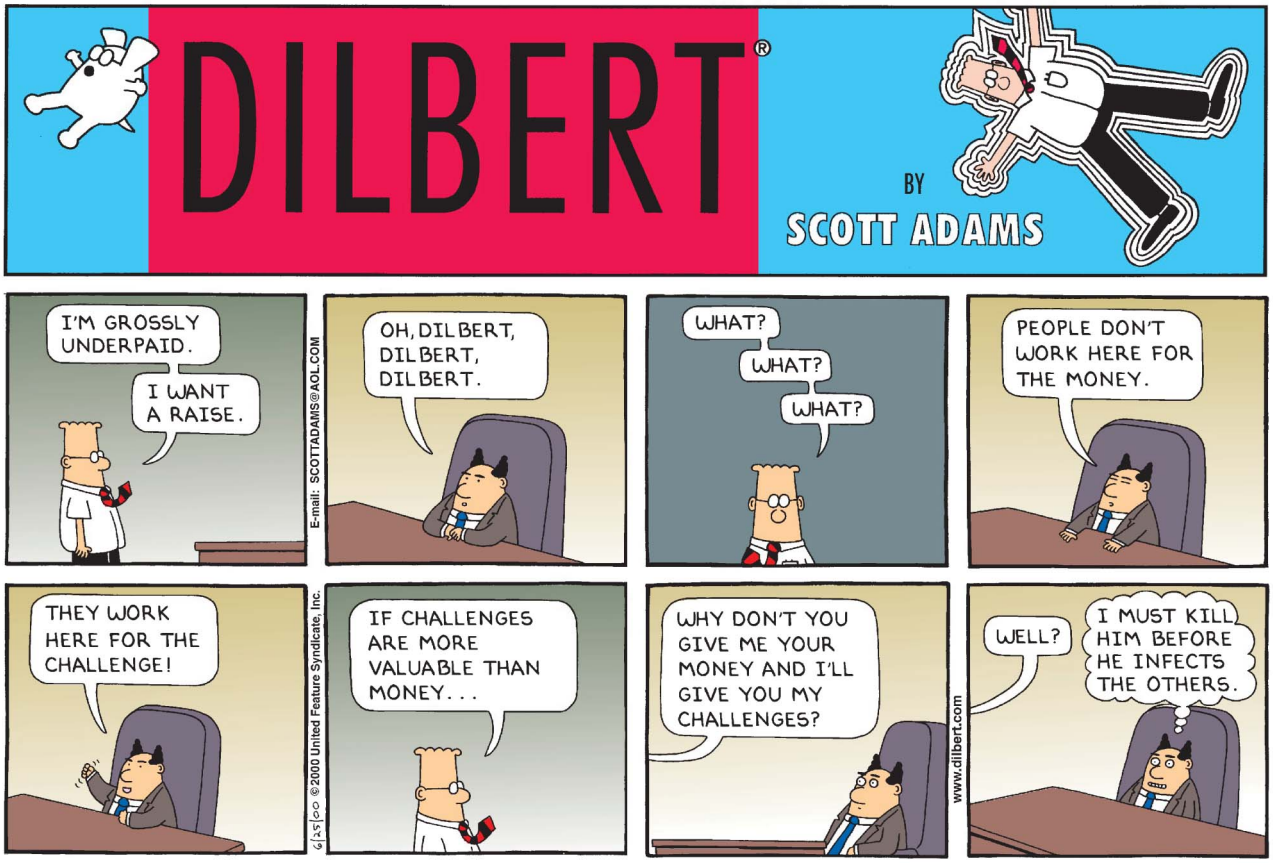
JUNE 27 Application deadline for the grant, **1000 GENOMES PROJECT DATASET ANALYSIS**. This NIH funding will support analysis of the full dataset from the project, including evaluating allele frequency distribution and signals of natural selection, producing additional data types, evaluating the strategies used to develop the dataset, and developing additional tools.

JULY 2 Application deadline for the **NLM APPLIED INFORMATICS GRANTS**. These grants will support translational efforts to bring useful clinical and biomedical research information into practice.

JULY 6 Application deadline for the **PROTEOMICS IN AUDITORY DEVELOPMENTAL AND DISEASE PROCESSES** grant.

Blunt End

HUMOR, WE HOPE



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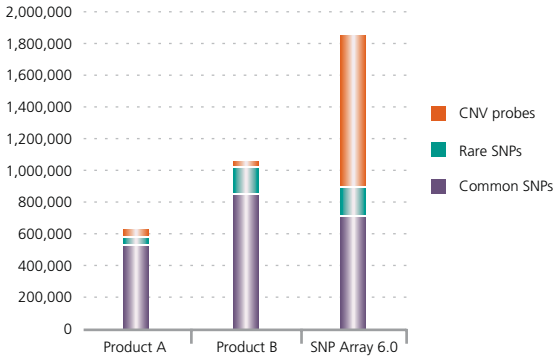
Number of SNPs and CNV probes across competing genotyping arrays in CEU



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