TheScientist

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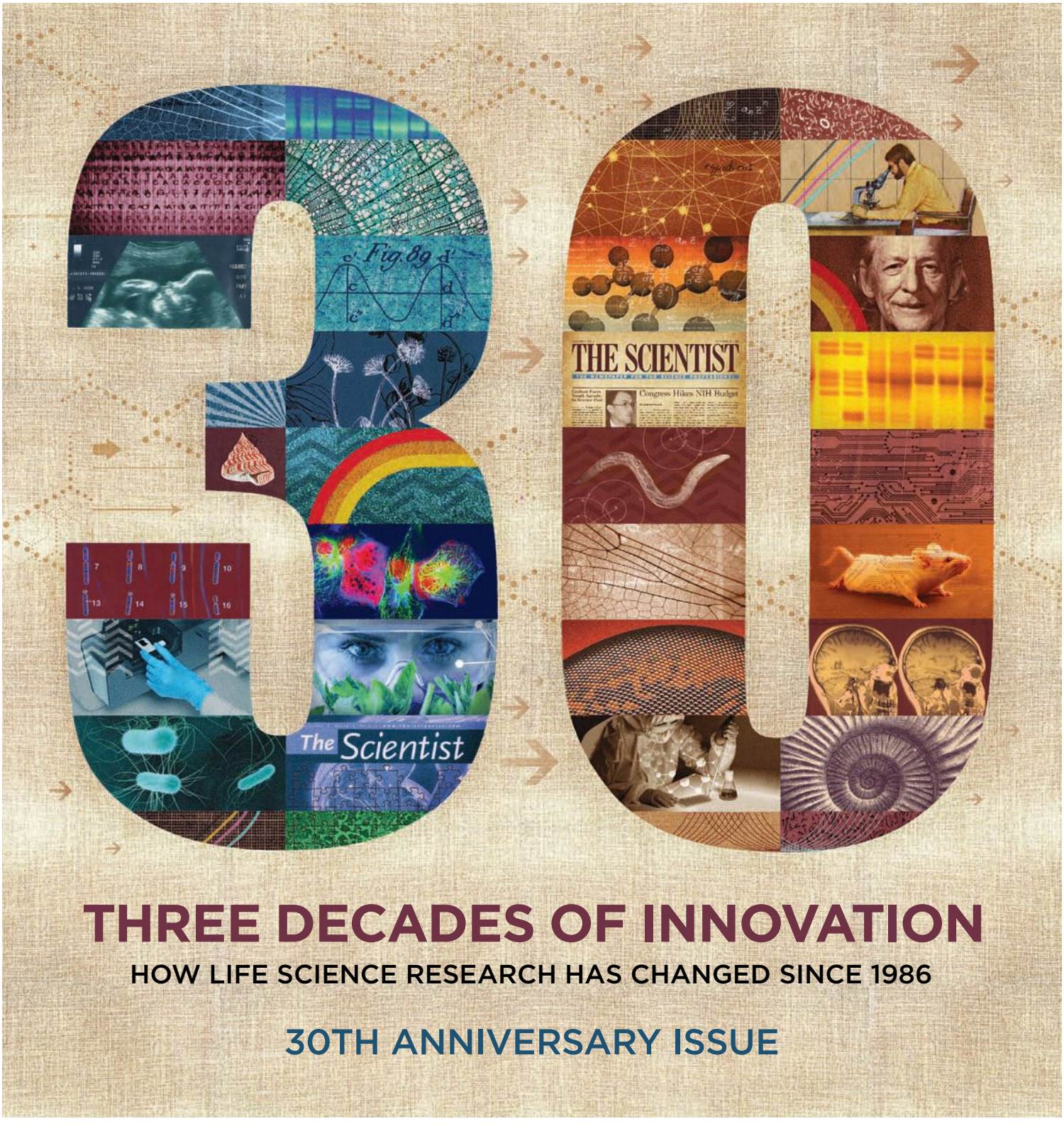

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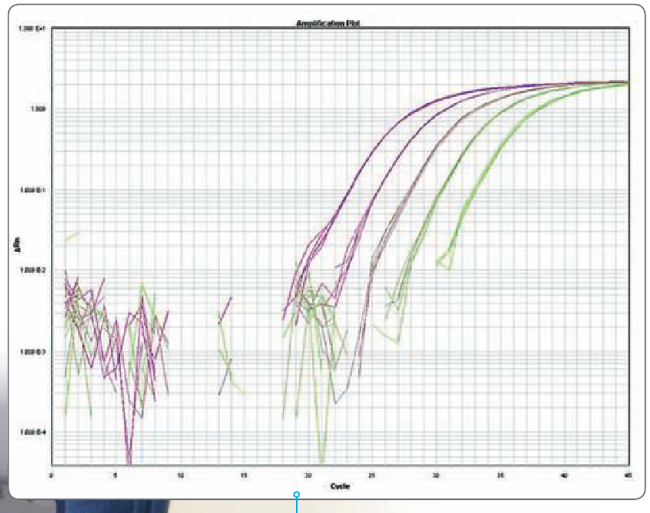

Standard Amplification Curves for COX gene. Standard cDNA samples were serially diluted 5-fold and amplified in 5 replicates (dilutions left to right: 5x, 25x, 125x, 625x and 3125x).

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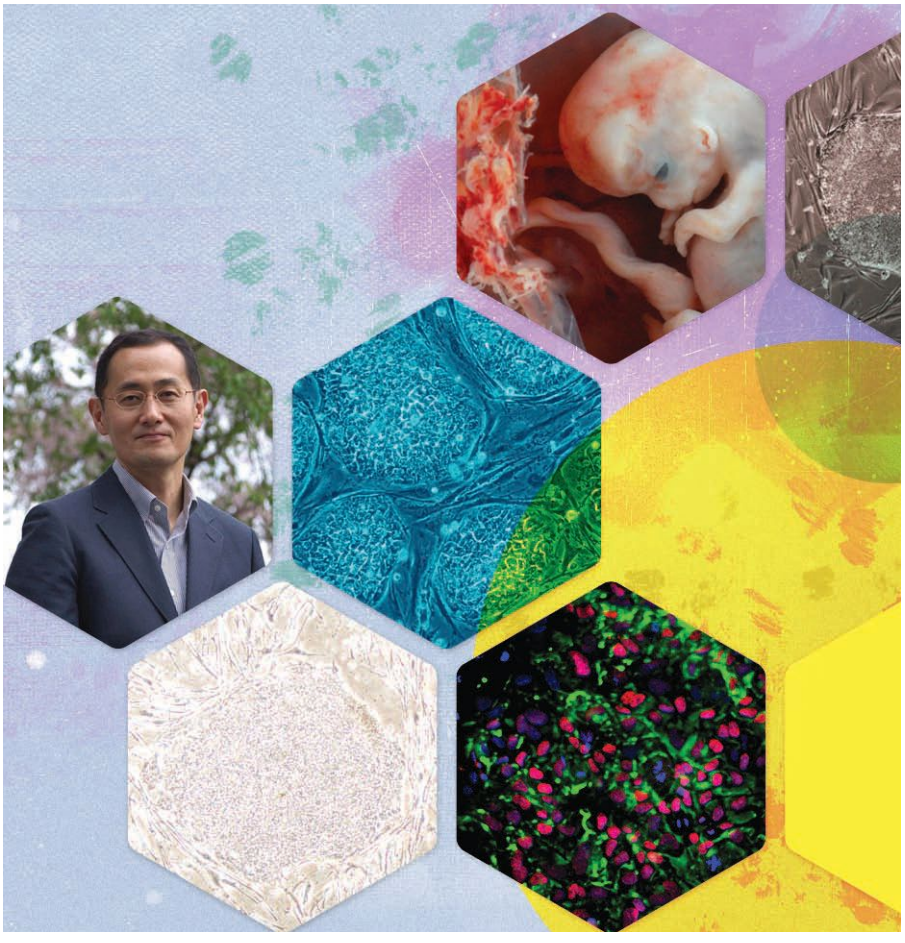

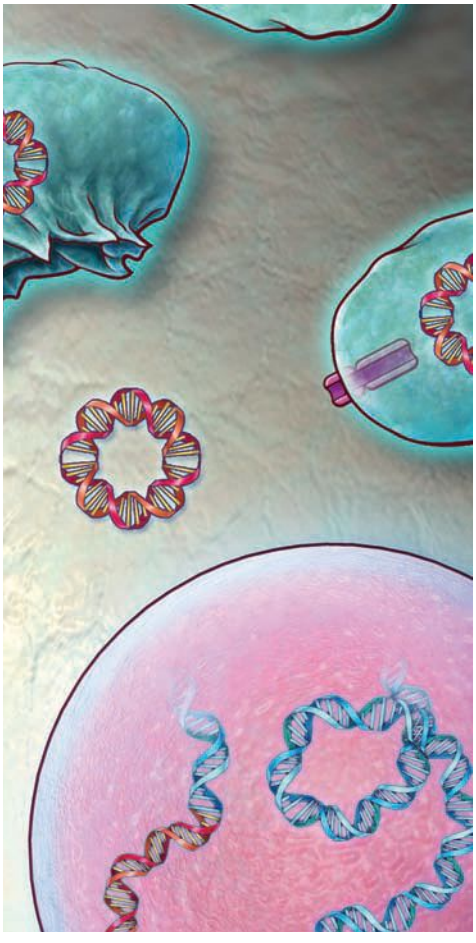

Features

ON THE COVER: ILLUSTRATION BY MARTIN O'NEILL

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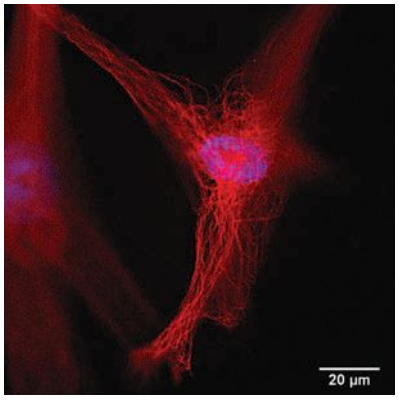

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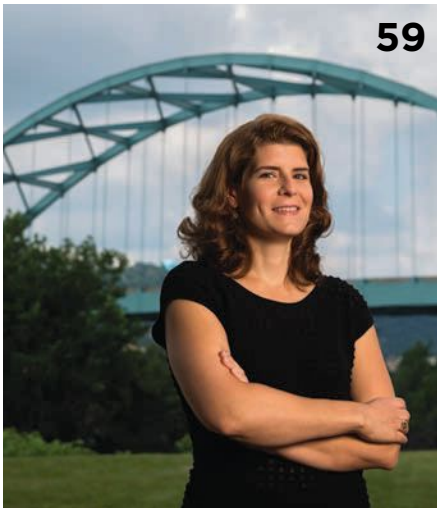
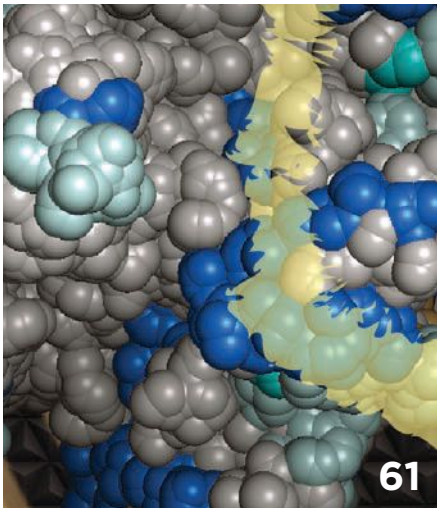


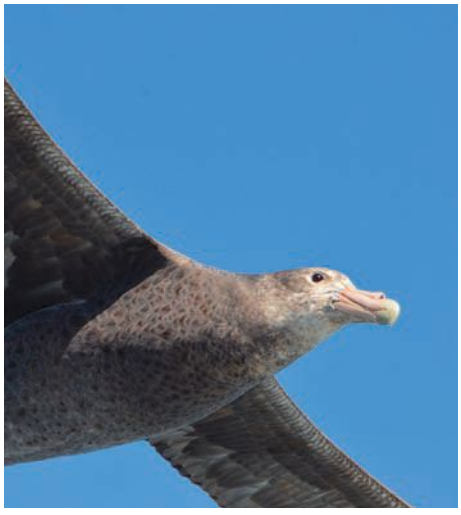


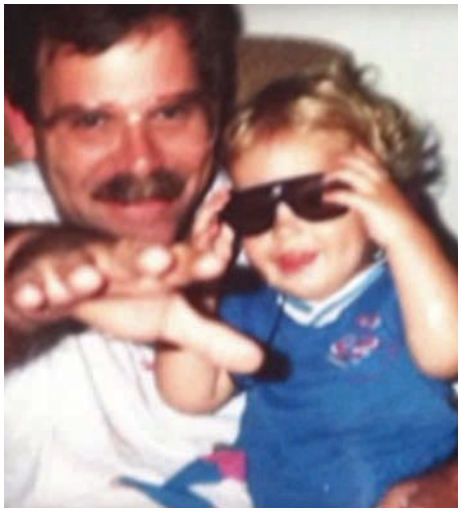
CORRECTIONS:

"The Challenges of Rare-Disease Research" (*The Scientist*, September 2016) incorrectly stated that Hamed Jafar-Nejad was unaware that jagged1 was mutated in Alagille patients. In addition, Heather Etchevers received funding from Nevus Outreach, Inc., not Naevus Global. Finally, rare diseases in the U.S. are defined as those that affect fewer than 200,000 people, not fewer than 1 in 200,000.

The Scientist regrets the errors.

Online Contents





THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Church on the Late Show
Harvard biologist George Church talks with Stephen Colbert about gene therapy, aging, and reviving the woolly mammoth.

VIDEO
Extreme Birding
Oregonian Noah Strycker broke the world Big Year birding record when he saw 4,342 species in 2015.

VIDEO
Saving Jon
Meet the researcher/entrepreneur who started a nonprofit that seeks to solve the science behind a rare disease that threatens her younger brother's life.

AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

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HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Immunity and the brain
- The human virome
- How to map the brain with DNA barcodes
- Using social media in research
- Plus: Annual Salary Survey

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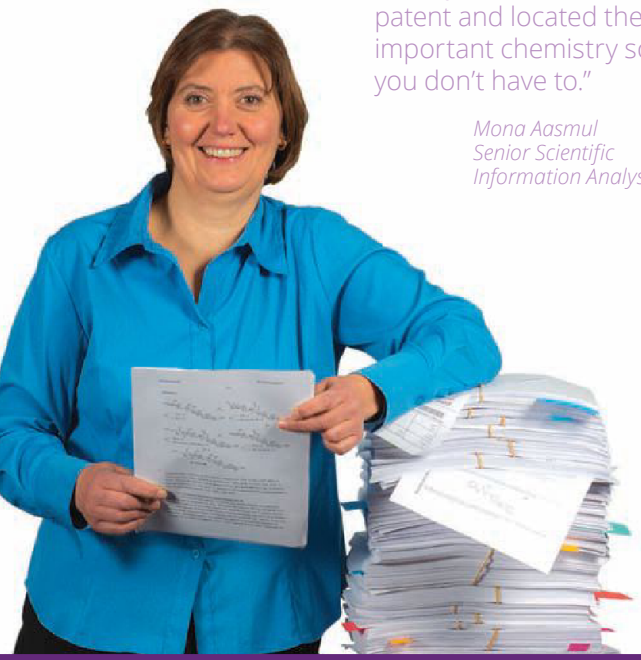

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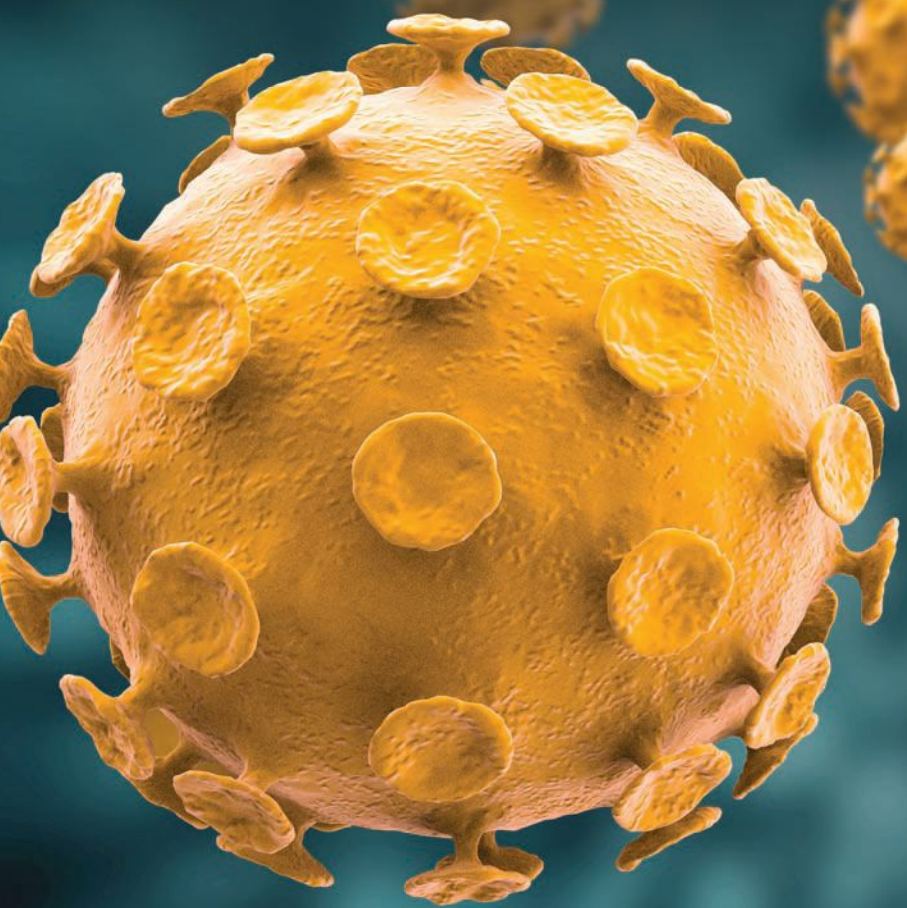

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Contributors

Kelly Robinson recalls learning early in high school that some human cells can divide countless times, "but somehow their genetic information stays the same." That "somehow" captured her interest, first because it seemed fantastically improbable, then later because of its scientific draw. Robinson earned a degree in genetics at Clemson University and is working on her PhD in molecular medicine at the University of Maryland, Baltimore. While helping to pioneer research on horizontal gene transfer from bacteria to humans, Robinson felt the excitement of discovery, "getting to figure it all out and piece it all together." But she also started to notice how hard it was for her and other scientists to share their work with the public, especially on platforms such as Facebook. She now plans to pursue work in outreach, education, or science policy after finishing her PhD in the spring of 2017.

Julie Dunning Hotopp's research has often put her at odds with scientific dogma, but she says thinking outside the box is just part of her job. When she and her colleagues started looking for bacterial DNA fragments in the *Drosophila* genome, "we had this experiment that just wouldn't work, wouldn't work, wouldn't work," Dunning Hotopp recalls, until she suggested something a colleague deemed impossible. What if the entire bacterial genome was there inside the fly genome? "We couldn't wrap our minds around it." Her hunch proved correct, and Dunning Hotopp would go on to document lateral gene transfer events in many invertebrate groups.

She got her start working in a research lab as an undergraduate and went on to earn a PhD in microbiology and molecular genetics from Michigan State University in 2002. She began her work with *Drosophila* during a postdoc at the Institute for Genomic Research and now teaches at the University of Maryland School of Medicine.

Robinson and her PI, Dunning Hotopp, delve into the possibility and consequences of horizontal gene transfer from bacteria to people in "Bacterial Cut-and-Paste" on page 46.

As **Catherine Coombes** was taking her A-levels in Harlow, England, in the 1990s, genetically modified organisms began grabbing headlines. From then on, she was enthralled with biotechnology. Coombes earned a bachelor's degree in the subject at the University of Leeds, graduating in 2000. After working in the scale-up of active pharmaceuticals, she became interested in how technologies are patented and completed a master's in intellectual property law at the University of London. Coombes, who began practicing law in 2004, joined the law firm HGF Limited in June 2015. There, she handles cases concerning immunology, biopharmaceuticals, and more—including the use of CRISPR in bacteria and gene editing. "I love my job," she says. "I think it's absolutely fascinating."

Coombes has been following the CRISPR patent battle unfolding in Europe with avid interest. In "Cautionary Tale from CRISPR" (page 27), Coombes uses the acrimonious CRISPR patent clash to give scientists some tips on how they might avoid such battles. "It doesn't have to be like that," she says. "You just have to have a little foresight earlier on."

Bruno Lemaitre grew up in northern France in the 1970s. "I collected stones, observed stars, and caught insects—I was a naturalist," he says. As a graduate student at the Pierre and Marie Curie University in Paris, his interests turned to *Drosophila* genetics. After earning his PhD with Dario Coen in 1992, Lemaitre moved to the lab of Jules Hoffman at the National Center for Scientific Research (CNRS) in Strasbourg, France, where he investigated the genetics of the *Drosophila* immune system. Lemaitre was a major player in the discovery of the Toll receptor's role in fruit fly immunity, for which Hoffman shared a Nobel Prize in 2011. In 1998, Lemaitre set up his own genetics lab at CNRS in Gif-sur-Yvette, France. And in 2007, he migrated to the École Polytechnique Fédérale de Lausanne in Switzerland.

Recently, Lemaitre has developed an interest in the study of personality and its influence in the scientific context, spurred by his complex relationship with his postdoctoral supervisor (discussed in his blog behinddiscoveries.com). Read "Truth and Power" on page 73, which is based on his recent book, *An Essay on Science and Narcissism*.

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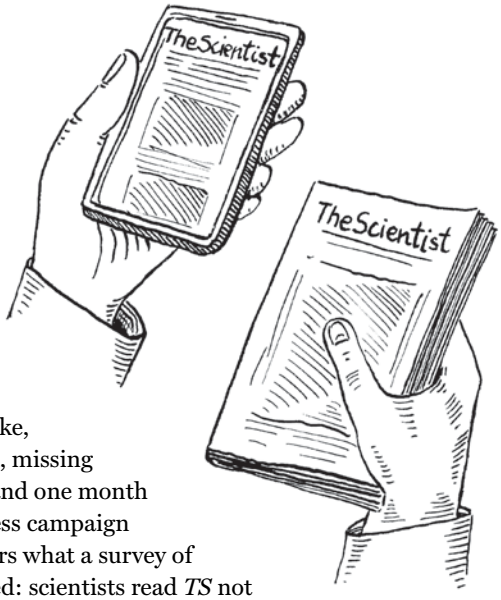
An Evolutionary History

Celebrating 30 years and a resurrection

BY MARY BETH ABERLIN



Five years ago, with much fanfare, we commemorated the 25th birthday of *The Scientist,* a unique science news and opinion outlet nursed into being in October 1986 by publishing entrepreneur and bibliometric visionary Eugene Garfield, founder of the Institute for Scientific Information (ISI). As I wrote in my editorial in that special issue, Garfield "launched the publication to disseminate information that he passionately believed was as important to scientists as what they read in research journals: news, opinions, and interviews about funding, ethics, politics, and other career-related issues, plus profiles, book reviews, and even pithy quotes."

From its origins as a 16-page biweekly newspaper, the print version morphed into a glossy monthly edition still bent on carrying out Garfield's vision. Not long after that 1986 inauguration, *The Scientist* added an online presence, the first science publication to make its full text available for free via the National Science Foundation Network, a precursor of the Internet.

TS continued to evolve, enhancing its coverage with breaking news and a news blog; introducing a daily newsletter email; producing podcasts, videos, and animated and interactive infographics; and establishing a social media presence—all the while facing the same pressures experienced by all living organisms: competition, lack of resources, and a continually changing environment. In fact, as many readers may remember, just days after that celebratory 25th edition was published, the magazine suffered a mortal blow: the Science Navigation Group, full owners of *The Scientist* from 2009 to 2011, shut it down.

In evolutionary terms, *The Scientist* had gone extinct. Twenty-fifth anniversary article titles like ". . . And Many Happy Returns" and "Alive and Kicking" suddenly appeared wildly inappropriate. The issue was filled with articles in which leading life scientists—E.O. Wilson, Eric Kandel, George Church, Chad Mirkin, J. Craig Venter, and others—looked back 25 years and then ahead the same time span, predicting what the next quarter century might hold for their fields. But *TS* wouldn't be reporting on those seminal future events any more.

Or would it?

Just days after the shutdown, something truly un-Darwinian occurred. LabX Media Group, headed by Bob Kafato and headquartered in Midland, Ontario, stepped up to buy the publication. Phoenixlike, *The Scientist* returned, missing just one week online and one month in print. A new business campaign explained to advertisers what a survey of readers had established: scientists read *TS* not because they *have to* but because they *want to*.

To celebrate reaching our third decade, we bring you a double-length feature (page 32) dedicated to 30 years' worth of technical innovations that have allowed major-league advances in genome sequencing, microscopy, gene editing, neuroscience, and stem cell biology, most of which were unimaginable in 1986. I'm proud to say that the magazine's archives fully attest to attentive coverage of these innovations as they arose. Our Lab Tools column (two articles in every issue) continues this tradition, and for eight years running we have honored such technical advances through our Top 10 innovations competition.

To continue generating our award-winning content, *The Scientist* has not stopped evolving. We are constantly commissioning feature articles written by leading life-science researchers, taking the pulse of breaking research news, publishing enterprising news stories, spreading that news via diverse social media outlets (our Facebook page recently surpassed 2 million likes), reformulating our award-winning infographics to be optimally displayed both online and on mobile devices, conducting an ambitious array of webinars, and brainstorming special issues that cover hot life-science topics from many different angles, as originally espoused by Dr. Garfield.

So here we are, five years resurrected and going strong. Truly an evolutionary anomaly. ■

Editor-in-Chief
eic@the-scientist.com

ANDRZEJ KRAUZE

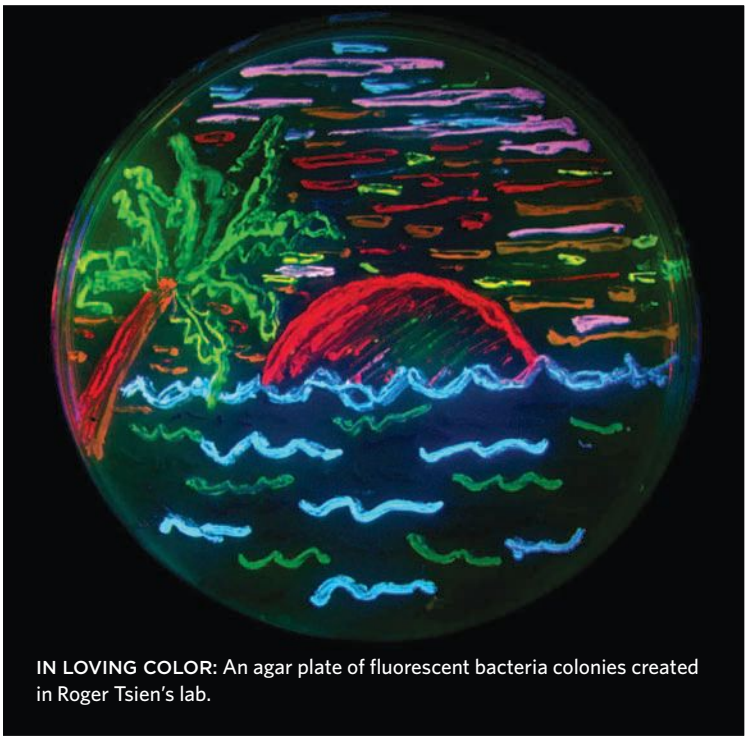
Speaking of Science

I've always been attracted to colors. Color helps make the work more interesting and endurable. It helps when things aren't going well. If I had been born colorblind, I probably never would have gone into this.

—Late biologist **Roger Tsien** of the University of California, San Diego, reflecting on his 2008 Nobel Prize for Chemistry, which he shared for his role in the development of green florescent protein (*San Diego Union-Tribune*, October 8, 2008)

Rarely are the smartest people the most creative too, but Roger was both.

—Nobel Laureate **K. Barry Sharpless** of the Scripps Research Institute in La Jolla, California, remembering biologist and fellow Nobelist Roger Tsien, who died in late August at the age of 64 (*Los Angeles Times*, August 31)



IN LOVING COLOR: An agar plate of fluorescent bacteria colonies created in Roger Tsien's lab.

TSIEN LAB, UNIVERSITY OF CALIFORNIA, SAN DIEGO

We take no sides between the traditional subscription model and the open-access model. We believe both of them can be done in a fair, open, clear, and lawful way. What we have a problem with here is people who are trying to benefit from the open-access model to scam people.

—**Ioana Rusu,** an attorney with the US Federal Trade Commission, on the legal complaint the FTC filed with alleged predatory publisher OMICS Group (*Inside Higher Ed*, August 29)

If you're looking for meaningful outcomes at the end of five years, then you might as well pull the plug now, because [NRMN] is going to fail. . . . Many of us are concerned that if NIH officials don't see results immediately, they will say, "Too bad, we tried. But that's the best we can do."

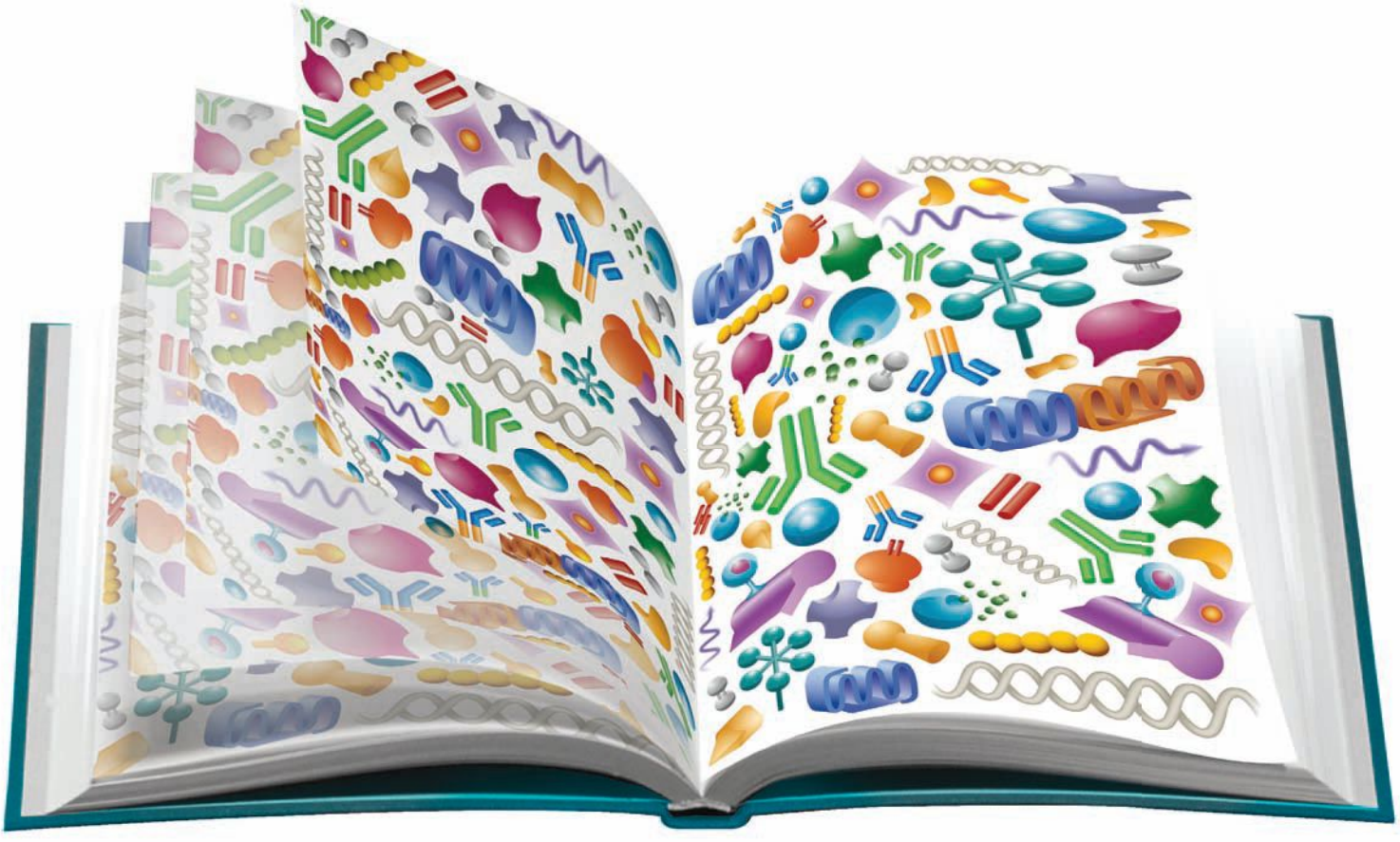
—**Donald Wilson,** cofounder of the Association for Academic Minority Physicians, commenting on the National Institutes of Health's new five-year, $22 million National Research Mentoring Network (NRMN), designed to increase diversity in the biomedical workforce (*Science*, September 2)

Brain size has increased about 350 percent over human evolution, but we found that blood flow to the brain increased an amazing 600 percent. We believe this is possibly related to the brain's need to satisfy increasingly energetic connections between nerve cells that allowed the evolution of complex thinking and learning.

—Australian biologist **Roger Seymour** of the University of Adelaide, in a statement on his team's recent study published in *Royal Society Open Science* (August 31)

Mechanical interventions included genital installation of large quantities of iodine solution instilled by urethral or vaginal catheters, or "hot boxes" where a person's body was put in a box to 43 °C to try to kill off the organism and not the host. A return to this preantibiotic era is becoming an increasing possibility unless we slow down the rate of resistance or develop new drugs soon enough.

—**Vanessa Allen,** chief of medical microbiology at Public Health Ontario in Toronto, on the new World Health Organization guidelines indicating that antibiotics to successfully treat gonorrhea are dwindling (*Science*, August 30)



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Notebook

OCTOBER 2016

I SPY: A Buller's shearwater (*Puffinus bulleri*) like this one pushed birder John Weigel beyond the prior American Birding Association record, set in 2013.

Breakneck Birding

It's been three years since Neil Hayward, a Boston-based biotech consultant on a sort of sabbatical from working life, broke a 15-year-old North American birding record. On December 28, 2013, with just three days left in his so-called big year, Hayward spotted a great skua out over the Atlantic Ocean, making the bird the 749th species he had seen and photographed that year—one more than revered birder Sandy Komito tallied in 1998.

It was a spectacular eleventh-hour finish to a year spent crisscrossing the continent. But this year, it would take only seven months for birders to best Hayward's record. In July, a gray partridge in Washington State and a Buller's shearwater in California gave birder John Weigel, an American expat turned Australian, his official 749th and 750th species of 2016.

Weigel, a record-holding bird spotter in his adopted country, knew from the beginning of the year that weather patterns were working in his favor. "It became apparent there were super numbers of rarities coming into the southern part of the U.S. near Mexico and Asian migratory species coming into Alaska," he says. "That's when I realized I could break that record . . . if the El Niño hangs in."

So Weigel set out to take on North America—specifically, a region designated by the American Birding Association (ABA) that stretches from Alaska to Florida and encompasses everything in between, plus 200 miles offshore.

Since surpassing Hayward, Weigel has added at least a dozen more birds to his list, with months to go before the year is up. When I caught up with him over the phone late this summer, he was getting ready to head out on a couple of boat

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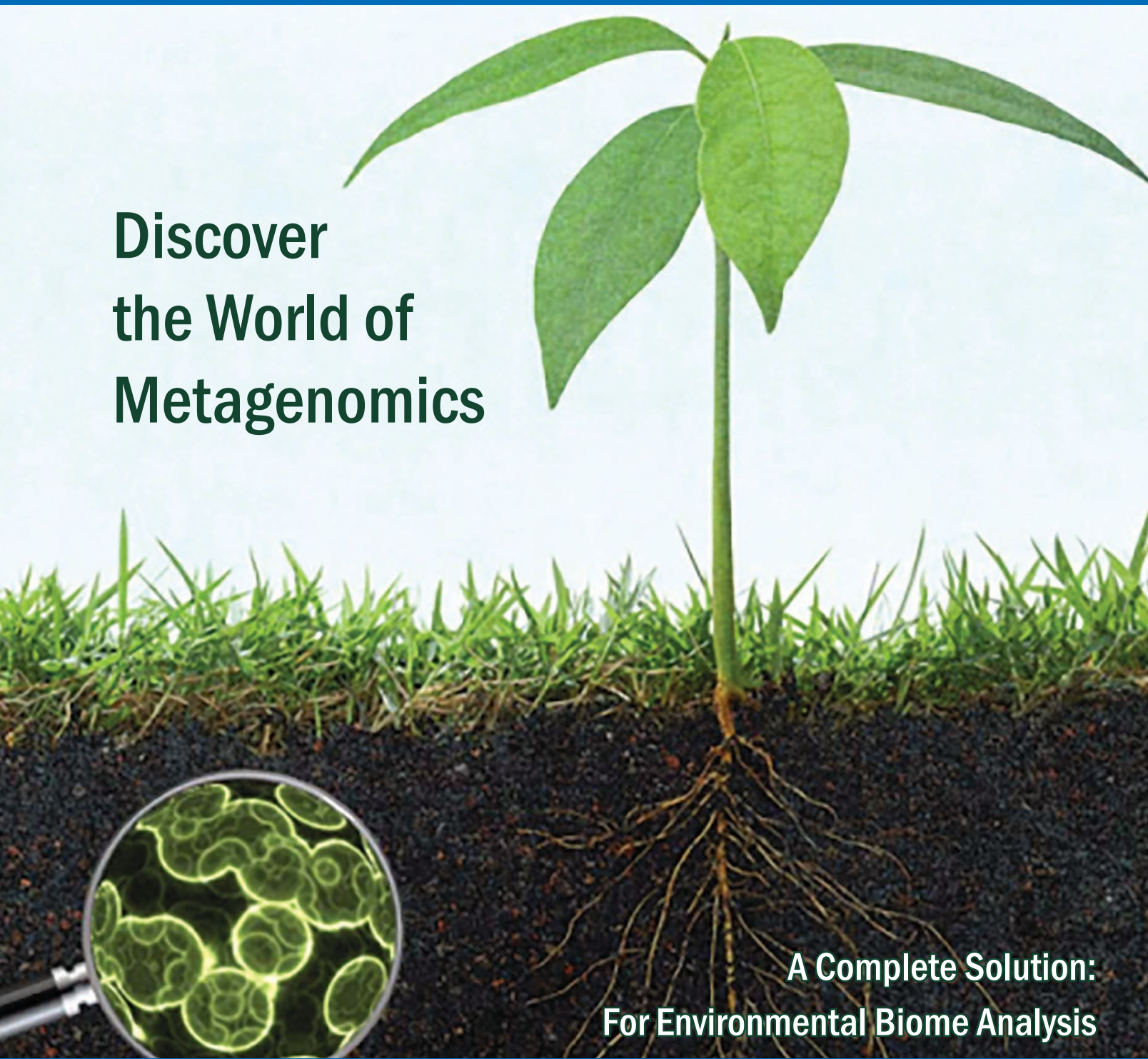

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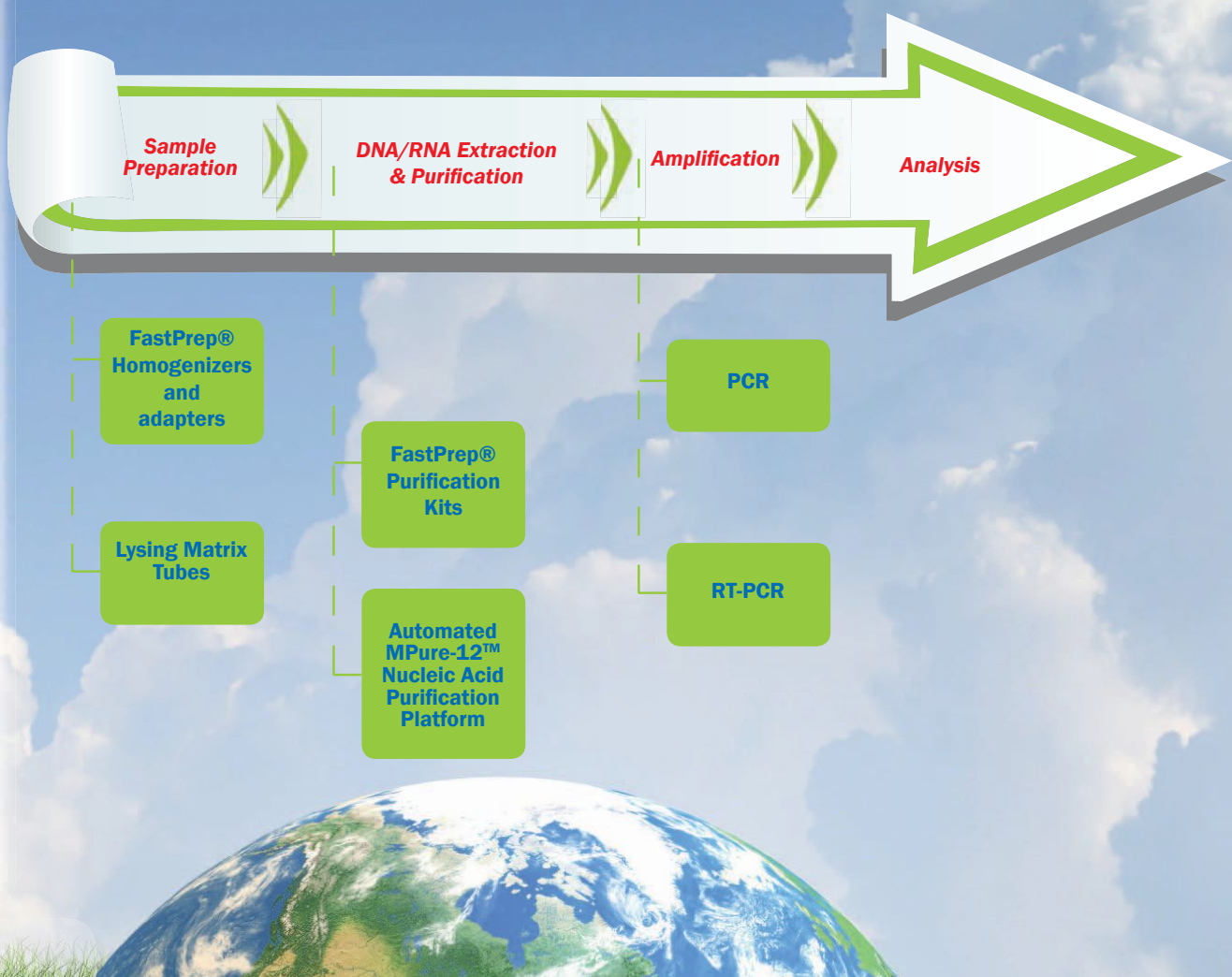

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FastPrep-96™

- **High Throughput:** Process up to 192 samples simultaneously in 2 x 96 deep well plates.
- **Exceptional Versatility:** Easily interchangeable adapters available for 2 x 96 deep well plates, 96 x 2 ml tubes, 48 x 4.5ml tubes, 20 x 15 ml tubes, 8 x 50 ml tubes and 2 x 250 ml bottles.
- **Modified Linear Motion:** Eliminates the need to reorient plates mid-cycle.

Cat.No: 116010500

DNA/RNA Extraction - FastPrep® Kits

DNA and RNA isolation from any environmental sample!

- Rapid and reproducible sample lysis and purification process.
- No cross-contamination with closed lysing matrix tubes.
- Increased yields of high-quality DNA and RNA.
- Integrity and size of DNA and RNA are retained.
- Ready-to-use in downstream applications.

FastRNA™ Pro Soil-Direct Kit

50 preps - Cat No. # 116070050

RNA from soil

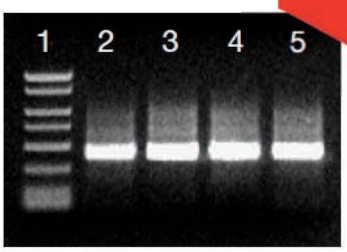
- Up to 500 mg of soil or other environmental sample.
- Lysing Matrix E tubes included for thorough sample lysis with a FastPrep instrument.
- 2 levels of purification to remove efficiently humic acids and other inhibitors.
- Cellular RNases are inactivated during homogenization to prevent RNA degradation.

Clean RNA for Uninhibited RT-PCR.

FastRNA Pro Soil Kits utilize unique Lysing Matrix E tubes and the power of FastPrep instruments to quickly disrupt all soil organisms. This process releases RNA into the protective RNApro™ lysis buffer faster than other lysis methods, efficiently inactivating endogenous RNases during homoge¬nization, minimizing RNA degradation.

A two-step purification includes a chemical extraction followed by a silica-based bind, wash, elute process that removes humics and other polyphenols as well as polysaccharides, resulting in inhibitor-free RNA for immediate use in RT-PCR and other downstream processes.

Two different kits offer flexibility to isolate RNA directly from soil samples, or indirectly by floating and filtering soil organisms first, allowing for processing larger soil amounts in the case of low-abundance or wet soil samples.



RT-PCR of Fungal Gene from Total RNA Isolated from Soil Samples with the FastRNA® Pro Soil-Indirect Kit. Approximately 40% of the RT-PCR reaction was loaded on to a 0.8% agarose gel. Lane 1: 150bp – 2kb marker, Lane 2: Soil #1, Lane 3: Soil #2, Lane 4: Soil #7, Lane 5: Soil #10.

FastRNA™ Pro Soil-Indirect Kit

50 preps - Cat No. # 116075050

RNA from soil supernatant

- Up to 1 g of soil or other environmental sample.
- Initial separation of microorganisms and other biological specimens from soil .
- Lysing Matrix E tubes included for thorough sample lysis with a FastPrep® instrument.
- Removal of PCR inhibitors and inactivation of cellular RNases during the homogenization step.

FastDNA™ Spin Kit for Soil

50 preps - Cat No. # 116560200

BEST SELLER!
Cited in more than 500 publications

Isolation of Pure Genomic DNA

- Up to 500 mg soil or other environmental sample.
- Lysing Matrix E tubes included for thorough sample lysis with a FastPrep® instrument.
- DNA purification by a silica-based spin filter method.
- Efficient removal of humic acids and other PCR inhibitors.

FastDNA™ 50ml Spin Kit for Soil

10 preps - Cat No. # 116560600

Isolation of Pure Genomic DNA

- Up to 10 g soil or other environmental sample.
- 50ml garnet Lysing Matrix tubes included for thorough sample lysis with a FastPrep® instrument.
- DNA purification by a silica-based spin filter method (50 ml Spin filter tubes).
- Efficient removal of humic acids/ polyphenols.

FastDNA™-96 Soil Microbe DNA Kit

2 x 96 preps - Cat No. #119696200

Isolation of PCR-Ready Genomic DNA

- Up to 130 mg soil or other environmental sample.
- 96 deep-well plates with Lysing Matrix Y for sample lysis with the FastPrep-96™ instrument.
- Yield is typically 5 µg of total DNA eluted in 50-100 µl of elution solution.
- Eliminates completely polyphenols and humic acids.

FastDNA™ Spin Kit for Feces

50 preps - Cat No. #116570200

Isolation of PCR-ready genomic DNA from stool samples

- Up to 500 mg of feces.
- Lysing Matrix E tubes included for thorough sample lysis with a FastPrep® instrument.
- Typically isolates 10 µg- 20 µg of DNA from 500 mg of stool.
- Removes organic contaminates for downstream applications.

FastDNA-96™ Fecal DNA Kit

2 x 96 preps - Cat No. #119696400

Isolation of PCR-ready genomic DNA from stool samples

- Up to 80 mg wet or dry stool per well.
- 96 deep-well plates with Lysing Matrix Y for sample lysis with the FastPrep-96™ instrument.
- Yield is typically 5 µg of total DNA eluted in 50-100 µl of elution solution.
- Purification process that eliminates completely PCR inhibitors.

FastPrep® extraction Kits have been specifically designed for the efficient recovery of inhibitor-free DNA from wide range of tough-to-lyse environmental samples.

Application notes available at
www.mpbio.com/FastPrepLibrary

Sample Preparation - Unique Adapters & Lysing Matrix

FastPrep® instrument adapters

Many interchangeable adapters for any sample size and extraction temperature.

Room Temperature Adapters

FastPrep-24™ 5G

Description	Cat.No
QuickPrep 24 x 2 ml	116002512
HiPrep 48 x 2 ml	116002527
TallPrep 24 x 4.5 ml	116002540
TeenPrep 12 x 15 ml	116002526
BigPrep 2 x 50 ml	116002525

FastPrep-96™

Description	Cat.No
2 x 96 deep well plates	119696168
QuickFlex 96 x 2 ml	116010570
TallFlex 48 x 4.5 ml	116010580
TeenFlex 20 x 15 ml	116010560
BigFlex 8 x 50 ml	116010550
LargeFlex 2 x 250 ml	116010590

Metal Adapters

FastPrep-24™ 5G

Description	Cat.No
All Metal QuickPrep 24 x 2 ml	116002545
All Metal BigPrep 2 x 50 ml	116002547

Cryogenic Adapters

FastPrep-24™ 5G

Description	Cat.No
CoolPrep 24 x 2 ml	116002528
CoolTeenPrep 6 x 15 ml	116002530
CoolBigPrep 2 x 50 ml	116002531

FastPrep® Lysing Matrix - Tailored to environmental samples.

The use of MP Biomedicals Lysing Matrix E & Y in combination with FastPrep® Instruments result in complete and quantitative lysis and thus higher yields of DNA and RNA.

Lysing Matrix E and Y tubes are designed to lyse all microorganisms including difficult sources such as eubacterial spores and endospores, gram positive bacteria and yeast, plant and animal tissues present in environmental samples.

DNase/RNase FREE CERTIFIED

Description	Composition	Pack Size	Cat.No
Lysing Matrix E tubes 	1.4 mm ceramic beads, 0.1 mm silica beads and 4 mm glass beads.	50 x 2 ml	116914050
		100 x 2 ml	116914100
		500 x 2 ml	116914500
		25 x 4.5 ml	116974025
		25 x 15 ml	116934025
		10 x 50 ml	116954010
Lysing matrix Y 96-Well Plates 	0.5 mm Yttria-stabilized Zirconium Oxide Spheres	1 x 96 well plate	116960001
		10 x 96 well plate	116960010

Barcoded 96 well plates are available upon request.

www.mpbio.com/FastPrepFamily

DNA/RNA purification - Automated MPure-12™ Platform

MPure-12™

A True Walk Away System.

- Rapid purification of nucleic acids
- Magnetic bead processing
- Pre-packaged reagent kits

- **Fully automated** and integrated platform that offers cost and time savings. Process 1-12 samples.
- **Reproducibility, lot-to-lot consistency, scalability, ease-of-use & convenience**.
- **Highest quality & yield** of DNA & RNA for downstream applications.
- **No cross-contamination** of samples thanks to the unique design of the platform.
- **Minimized nucleic acid loss & degradation**.
- **Increased safety & efficiency** through a closed system

Description	Pack Size	Cat.No
MPure-12™ system	1 unit	117002200
MPure-12™ Bacterial DNA extraction kit	48 preps	117022600
MPure-12™ Viral/Pathogen Nucleic Acids extraction kit B	48 preps	117022130
MPure-12™ Cell & Tissue total RNA extraction kit	48 preps	117022160

Genomic Access & Purification Workflow

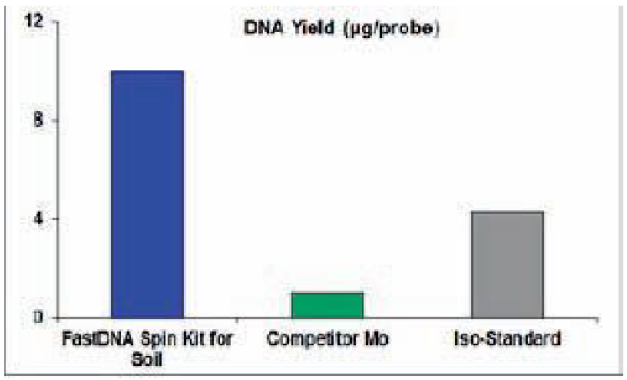
Cells to Nucleic Acids in ~45 minutes

Nucleic Acid Extraction

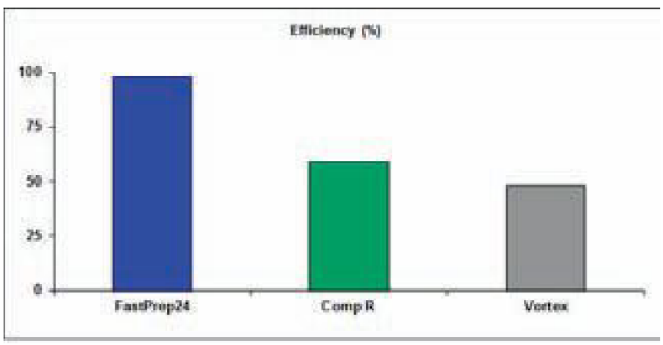
Data Proves Superior to Competition

Metagenomic studies involve isolation of nucleic acids from the entire biome of a given sample. Environmental or gut samples can present significant challenges in terms of sample preparation and subsequent isolation and purification. Typical soil, sludge and fecal samples exhibit variables such as, complex matrices with varying mechanical and rheological properties; a large diversity of biological materials including microorganisms, plant and animal tissue and other cells; and can contain innate PCR inhibitors and degrading enzymes; all of which combine to make processing procedures difficult to standardize. The FastPrep® System of sample prep instruments and isolation kits simplifies these procedures through automated, quantitative mechanical lysis of even tough Gram + bacterial spores and parasitic oocytes, and unique chemistry that flocculates and removes inhibitors followed by a simple, high capacity solid-phase silica, bind-wash-elute protocol.

Key Performance Indicators of Samples Processed with FastPrep-24™ and the FastDNA SPIN Kit for Soil

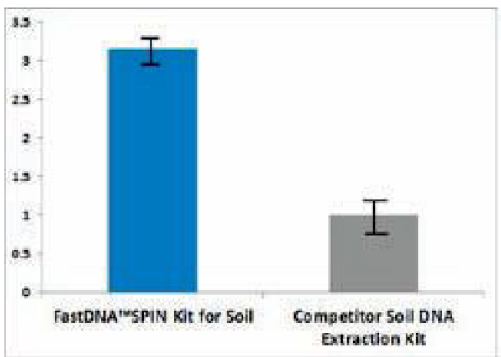


Total biome gDNA was extracted from equivalent soil samples using the FastDNA SPIN Kit for soil, a competitor's kit, and the ISO-Standard method.



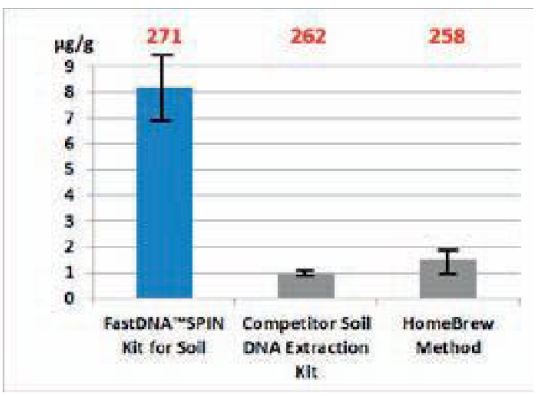
The FastDNA SPIN Kit for Soil was used to extract total biome gDNA with 3 different instruments to measure lysis efficiency. 3 equivalent samples in Lysing Matrix E

FastPrep vs Competitor
Bacterial 16S rRNA gene copies per ml of soil (x10^6)



Amplification of 16S rRNA genes using real-time PCR after DNA Isolation from soil samples. Data are calculated as the number of copies per milliliter of soil. Data are means (SEs) for six soil samples.

Biodiversity (total genera identified)



DNA yield (µg/g dry weight ± SD) from a fine-textured, alkaline , high organic content soil from deciduous forest leaf litter

www.mpbio.com

World's Leader in Soil Applications

Isolating DNA or RNA from soil without contamination by humic acids or other PCR inhibitors can be a challenge. The FastDNA™ SPIN Kit for Soil and FastRNA™ Pro Soil Kits used in combination with the FastPrep® instrument will help overcome any difficulties with complete lysis of all soil organisms including historically difficult sources such as eubacterial spores and endospores, gram positive bacteria, yeast, algae, nematodes and fungi, and isolation of pure DNA and RNA. Effective, efficient sample preparation is critical to succcessful downstream results.

DNA Extraction From Andisol, a Volcanic Ash Soil

DNA extraction from Andisol, a volcanic ash soil, is known to be very difficult because this soil has a complex matrix, including allophane, as a clay mineral. Soil properties such as high clay content contribute to high adsorption of DNA to soil particles. The combination of the FastPrep instrument and the FastDNA™ SPIN Kit for Soil used together with skim milk have demonstrated successful extraction of PCR-suitable DNA from recalcitrant soil samples like volcanic ash soil.

Materials

- FastPrep® instrument
- FastDNA™ SPIN Kit for Soil
- Skim milk (carrier minimizing adsorption of nucleic acids to soil)
- Sample: Andisol, volcanic ash soils

Protocol and parameters

1. Add the soil sample together with or without 40 mg skim milk per gram of soil to a Lysing Matrix E tube.
2. Add 978 µL sodium phosphate buffer to the sample in the Lysing Matrix E tube.
3. Add 122 µL MT Buffer.
4. Homogenize using FastPrep® instrument for 40 seconds at a speed setting of 6.0. m/s.
5. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.
6. Follow the FastDNA™ Spin Kit for Soil protocol for DNA purification from the homogenate.

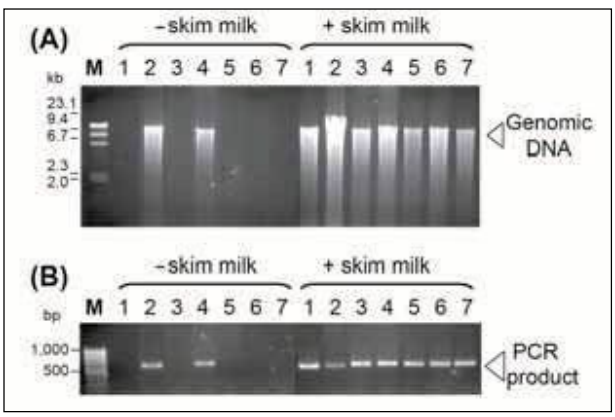
Chemical and physical properties of soils used for DNA extraction

Soil no	Origin	Soil taxonomy[a]	Soil texture	pH (H_2O)	Organic C content (g kg^{-1})	P retention (%)
1	Spinach field, Ibaraki	Dystric-Silic Andisol	light clay	5.46	83.419	83
2	Conserved forest, Ibaraki	Dystric-Silic Andisol	light clay	4.84	149.43	84
3	Apple orchard, Aomori	Silic-Eutrisilic Andisol (Dystric)	sandy clay loam	6.08	122.893	75
4	Vegetable field 1, Fukushima	Dystric-Silic Andisol	light clay	6.20	78.795	71
5	Vegetable field 2, Fukushima	Haplic-Dystric Cambisol	clay loam	6.02	23.239	65
6	Upland crop field, Kumamoto	Dystric-Silic Andisol	heavy clay	5.59	117.283	82
7	Paddy field, Kumamoto	Silic-Eutrisilic Andisol (Dystric)	heavy clay	6.38	119.425	91

a: According to the world reference base (WRB) for soil resources classification.

Results

DNA could successfully be extracted from Andisol soil samples with the FastDNA™ SPIN Kit for Soil and the addition of 40 mg of skim milk per gram of soil sample. PCR products of the expected size were amplified from all extracts with skim milk. Resultant extracts were suitable for PCR and no other purification procedures were needed.



Agarose gel electrophoresis of DNA and PCR products extracted from sample soils (1-7) FastPrep was used for extracting DNA from soils (A). PCR products from these extracts were amplified with bacterial 16S rONA universal primer set (338f and 907r) (B) when amended with (+) or without (-) 40 mg of skim milk g^{-1} soil. Numbers show soil samples listed in Table I. M: Molecular marker (A:λHind III digest, B: 100 bp ladder).

References:
Takada Hoshino, Y and Matsumoto, N, Skim Milk Drastically Improves the Efficacy of DNA Extraction from Andisol, a Volcanic Ash Soil. Japan Agri Res Quart, 2005, 39, 247 - 252.

Product Overview

The FastDNA™ SPIN Kit for Soil is designed to efficiently isolate bacterial, fungal, plant, and animal genomic DNA from soil and other environmental samples. The FastRNA™ Pro Soil Kits are designed to efficiently isolate total RNA from organic material found in soil samples and soil sample supernatants.

Superior Performance in Microbial Applications - Bacteria

Extraction of labile enzymes from gram-positive bacteria with the FastPrep® System

To demonstrate the capacity of the FastPrep System to deal with otherwise difficult to lyse bacteria, we chose to study two gram-positive bacteria. These two species, Bacillus amyloliquefaciens and Staphylococcus aureus 3A, are extremely resistant to classical cell rupture by methods such as sonication. These bacteria are used as a source for the production of two restriction enzymes, Bam HI and Sau 3AI respectively. The instability of these enzymes is well-known, and special conditions during purification and storage are necessary to maintain their biological activity. It is clear that these enzymes have to be purified under the most stringent conditions in order to prevent denaturation by factors such as proteases, heat, chemical agents, and others.

Materials and Methods

Lysis of Bacillus amyloliquefaciens with a standard lysing procedure involving sonication was compared with a modified FastProtein Blue Kit protocol (Cat N°: 6550-400).

Staphylococcus aureus 3A with its extremely thick cell wall is resistant to sonication. Normally, for successful large-scale extraction of production size volumes of Sau 3AI, use of the French Press is necessary; alternatively, protoplasts can be prepared. Here we utilize a small-scale sonication protocol for comparison with a modified FastProtein Blue Kit protocol.

Cell density

- Sonication: Bacterial suspensions of 0.2 g wet weight (w/w) and 0.15 g (w/w) per mL of buffer for Bacillus amyloliquefaciens and Staphylococcus aureus 3A, respectively.
- FastPrep: Bacterial suspensions of 0.1 g (w/w) and 0.4 g (w/w) per mL and 0.15 g (w/w) per mL of buffer for Bacillus amyloliquefaciens and Staphylococcus aureus 3A, respectively.

Disruption

- Sonication: Bacteria are disrupted at 50% maximum intensity (large tip) for Bacillus amyloliquefaciens and 20% maximum intensity (small tip) for Staphylococcus aureus 3A with a Branson Sonicator B30. Temperature is maintained at 4°–5° C by cooling in an ice salt water bath. Sonication was continued for 10 min in 40 s bursts for Bacillus amyloliquefaciens and 60 s in 5 s bursts for Staphylococcus aureus 3A.
- FastPrep: The FastProtein Blue matrix was used. Tubes containing the lysing matrix and sample were prechilled at 4° C then mixed. Samples are homogenized with the FastPrep® instrument at speed 6.0 for 40 s for Bacillus amyloliquefaciens and at speeds 4.0 and 6.0 for 20 s and 40 s respectively for Staphylococcus aureus 3A. The tubes were returned to the ice bath. Homogenization and chilling was repeated for all time points.

At each time point a 50 µl sample was taken, centrifuged for 5 min at 4°C in a benchtop centrifuge and tested for OD_{260} and activity.

Results

1. Lysis

Bacillus amyloliquefaciens. Figure 1 shows that the optimal lysis of cells is achieved after 3 x 40 s treatments in the FastPrep instrument at speed 6.0 (red line), while equivalent lysis by sonication required 9 x 40 s bursts for equivalent quantities of cells (OD_{260} of sample diluted 100 times) (blue line). This means that for Bacillus amyloliquefaciens, the FastPrep produces a more complete lysate in approximatively one-third of the time. This time-saving feature also reduces the amount of time the extracted material is exposed to the denaturating conditions required to lyse the bacteria.

Staphylococcus aureus 3A. Figure 2 indicates that optimal lysis of cells is achieved after 3 x 40 s treatments in the FastPrep instrument compared with 10 x 5 s bursts (every other time point shown) of sonication for the equivalent quantity of cells (OD_{260} of sample diluted 200 times). This shows that for Staphylococcus aureus 3A, 3 x 40 s treatments was optimal for the release of extracted material (green line), while even ten 5 s sonication bursts released almost no material (blue line). The relative ease of lysis for the FastPrep® method is clearly more effective than sonication, and is much faster and easier to perform than the classical French Press technique (data not shown).

Figure 1: Bacillus amyloliquefaciens

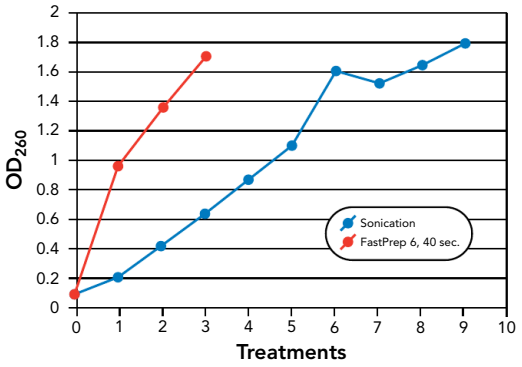
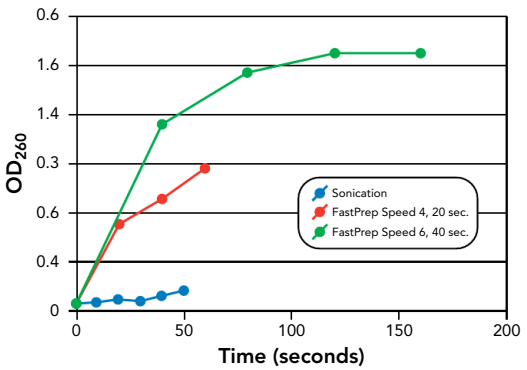


Figure 2: Staphylococcus aureus 3A

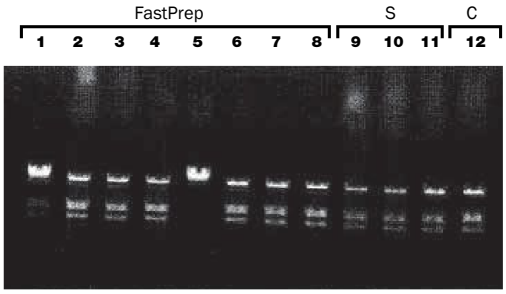


2. Activity testing

Bacillus amyloliquefaciens. The activity of lysed samples from Bacillus amyloliquefaciens was tested on λ DNA. 10 µL of each supernatant was diluted five times in Bam HI storage buffer and then 2 µL was mixed with 1 µg of DNA in a 50 µL reaction volume and incubated at 37 ° C for 30 min. The reaction was then observed by agarose gel electrophoresis followed by ethidium bromide staining (see Figure 3). Results show that all **FastPrep®** samples retained Bam HI activity, even at the shortest processing time of 40 s.

Staphylococcus aureus 3A. The activity of lysed samples from Staphylococcus aureus 3A was tested on λ DNA. 2 µL of each supernatant was mixed with 0.6 µg of DNA in a 25 µL reaction volume and incubated at 37 ° C for 30 min. The reaction was then observed by agarose gel electrophoresis followed by ethidium bromide staining. No restriction endonuclease activity was observed from any of the sonicated samples, and limited activity was observed from samples processed at speed 4 in the **FastPrep® instrument**. However, samples that were processed at speed 6 showed increased activity, with the highest level of activity at 4 x 40 s (data not shown).

Figure 3: Aqarose gel electrophoresis with ethidium bromide staining



Lanes 1 to 8 correspond to the samples processed in the FastPrep: 1 to 4 are at 0.4 mg/ml and 5 to 8 at 0.1 mg/ml. 1 and 5 at time 0, 2 and 6 at 40 s, 3 and 7 at 2 x 40s, 4 and 8 are 3 x 40 s. Lanes 9, 10 and 11 correspond to sonication samples (S) taken at 4 x 40 s, 7 x 40 s and 9 x 40 s, respectively. Lane 12: I DNA cut by purified Bam HI (C).

Conclusion

These experiments clearly show that the **FastPrep® instrument** using **FastProtein Blue matrix** can be used to successfully extract unstable enzymes from gram-positive bacteria. Even in cases where sonication can release active materials (such as the Bacillus amyloliquefaciens experiments here), the lysing time can be reduced by approximately 60%. For samples like Staphylococcus aureus 3A that require longer and less efficient methods of lysis (such as French Press), the **FastPrep®** method offers clear advantages for extraction of active proteins.

Life isn't a Race to the Finish Line, It's a Drive to Scientific Discovery!

MP Research Grant Program

Analyte Isolation:

(Nucleic acids and proteins from difficult samples)

Bone • Insect • Plant • Bacteria • Soil

Support with FastPrep® instrumentation, Lysing Matrix and consumables

Applications are currently being accepted
Deadline to apply is September 30, 2016

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MP Biomedicals - *It's What's Inside That Counts!*

trips off the coast of California to see if he could sight a blue-footed booby. (He did.)

Even more remarkable than Weigel's accomplishments is the fact that he's not alone. There is another birder—a retired medical-staffing executive and nudist known by his pen name, Olaf Danielson—who has kept pace with Weigel. Aiming to break big year birding records requires months of travel—by boat, plane, car, and foot—oftentimes on short notice. "It's been a hideously expensive and difficult thing to get ahead of this guy," says Weigel.

Weigel grew up in Colorado, and his accent retains much of his American roots, while words such as "life" and "time" bear the vocal lilt of Aussies. He owns a zoo, the Australian Reptile Park, about an hour's drive from Sydney, and he uses his birding efforts to raise funds for a Tasmanian devil breeding program called Devil's Ark.

Danielson has been birding since his childhood in Wisconsin. "My second word was chickadee," he says. In 2013, Danielson set the record for the most bird species spotted while nude—594. "That was my weird year," he says, which included a frigid excursion hiking naked on Attu—the westernmost island of the Aleutians and a famed birding site.

Danielson had planned to devote 2016 to breaking Hayward's 2013 record. His business partner had bought him out, his grandmother fell ill, and he turned 50. It seemed like the right time to take on a big year. "I hadn't set a plan for competing with anyone else," he says, and he maintains that his efforts are not directed at out-birding Weigel. Still, he's publicly questioned Weigel's methods, writing on his blog in May: "Like the Devil, the devilbirder is everywhere all the time."

There are few people who log more than 700 bird species spotted in any given year. Not only does it take exceptional birding skill, but an enormous investment in time and money. Birders are sometimes chasing after a single individual that may only stick around in one area for a few days at a time.

"Some weeks you're lucky," says Danielson, "other weeks you fly all the way to California to see a marsh sandpiper—the only one this year in North America. But you should have been there yesterday." After missing the sandpiper, Danielson drove "helter skelter" to San Francisco Bay and missed another bird, then flew overnight to Key West, only to be a day late again. "There's nothing worse than dragging all over the place to see a bird, and you miss it." He estimates that by the end of the year he'll have spent about $75,000 chasing birds.

Those unfortunate episodes aside, part of what has made 2016 so successful is that some of these fleeting species have stuck around. Tufted flycatchers, for instance, have spent half the year in southeastern Arizona, when normally they pop up from Mexico only momentarily, says Nate Swick, manager of the American Birding Association's blog. He says this is the first year four people—the other two being Christian Hagenlocher and Laura Keene—will break the 700 mark.

If there was no one else this year, I'd be having so much fun. But I can't get through this year and be a bird short, can I?

—John Weigel, birder

Along with the weather, a few other developments have helped propel birders to this elite echelon. For one, the ABA list of species has expanded based on DNA analyses that have split a single species in two (and this has happened a few times). Danielson says birders have also seized upon cruise ship excursions up the Pacific Coast, helping add offshore species to their sightings.

Online resources have also given a huge boost to birders. In particular, eBird, a website run by the Cornell Lab of Ornithology and the National Audubon Society, allows birders to post their sightings in an easily searchable format. "We figured out very early on that birders plus competitive spirit equals more data," says Brian Sullivan, the eBird project leader. "The data is so dense in eBird now that you can get a likelihood of seeing a certain species at a certain site for every week of the year."

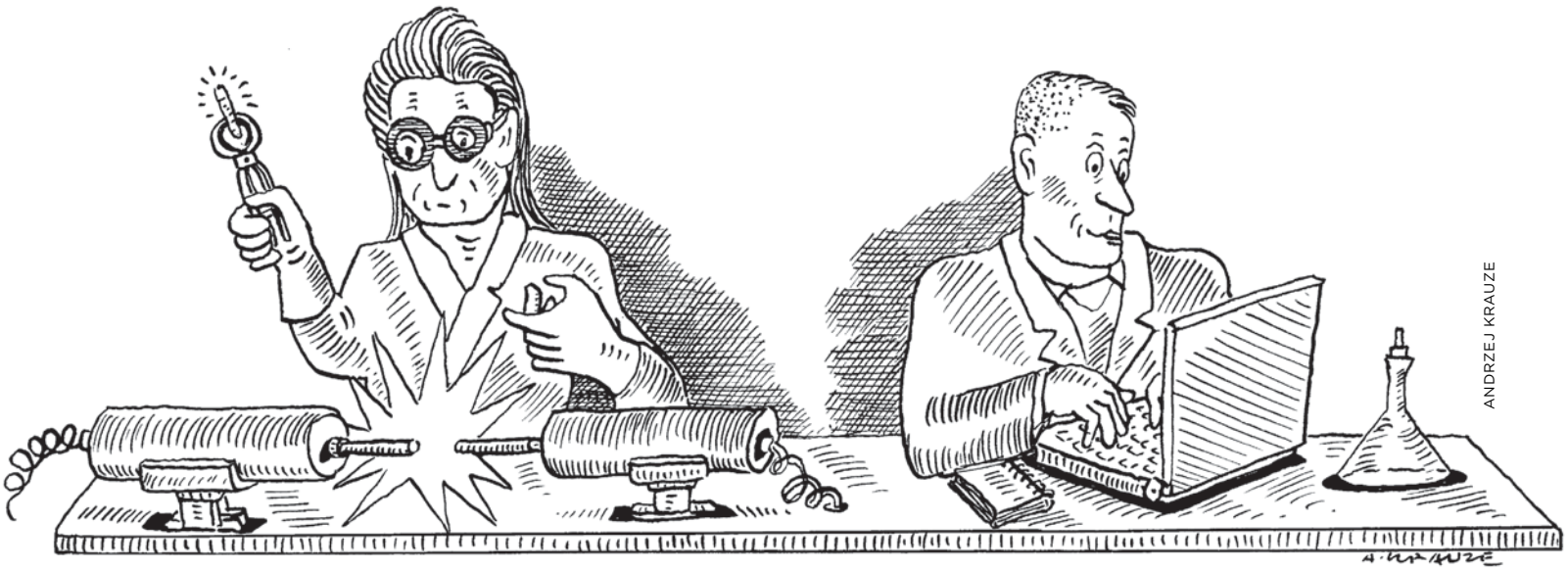
The goad of competition cannot be discounted in this year's race for a new record. For Weigel, it's no longer about beating Hayward, or going beyond what Sandy Komito would have tallied had he been working with the ABA's 2016 list of species (about 765, by Weigel's count). It's about beating Danielson. "It's a shame," Weigel says. "If there was no one else this year, I'd be having so much fun. But I can't get through this year and be a bird short, can I?" —Kerry Grens

Keep Calm and Pipette On

The public's view of the scientific endeavor often conjures up stereotypical imagery: a begoggled and lab-coated researcher sitting in a stark room operating ergonomically designed technology. But as anyone who has actually worked in a lab knows, that picture could not be further from the truth. Working experimentalists are generally more than familiar with foul smells, contamination mishaps, cluttered and clunky equipment, and space shortages. Since the dawn of science, the issue of safety in the confinement of the experimental arena has been a significant problem—even if it was not recognized as such.

The price of some of the most transformative scientific discoveries was the health of their discoverers. Beyond the famous case of Marie Curie-Skłodowska, Robert Bunsen, the inventor of the ubiquitous Bunsen burner, lost his eye in a lab explosion and nearly died from exposure to the arsenic compounds he had synthesized. High-ranking scientists attempting to isolate fluorine lost their lives in explosions or from poisoning. It is also rumored that Galileo permanently blinded himself by observing the sun through one of his telescopes.

Even at the beginning of the 20th century, health-related hazards were considered an almost inevitable conse-



quence of conducting science. To quote the brilliant organic chemist August Kekulé: "Who does not ruin his health by his studies, nowadays will not get anywhere in Chemistry." A lot has changed since Antoine Lavoisier sparked the "chemical revolution" more than 200 years ago. The last century has seen an increasing awareness of the risks of laboratory work, and the rapid expansion of biomolecular sciences towards the end of the millennium created never-before-encountered types of hazards, requiring new approaches.

The past 30 years have been thankfully free from many previously common but dangerous lab practices such as in-lab smoking, washing hands in benzene, and tasting reagents. One can see evidence of a new culture of lab safety in the layout of modern labs. Offices are typically completely separated from laboratory spaces, for example. This arrangement was rare in the mid-1980s, but is now common practice.

Lab managers have also grown more aware of the occupational hazards at the bench. High-quality, flame-resistant materials are now commonplace, and gas fixtures have largely disappeared from biological labs. Every aisle is now equipped with an emergency shower, there are designated areas for working with radiation, and separate sinks for laboratory work and hand washing.

Perhaps the most significant change in lab-safety protocols over the past three decades involves the practice of mouth pipetting. Veteran life scientists could likely compete on what was the most dangerous substance they have accidentally gulped while mouth pipetting. Even solutions of pathogenic cultures or radioactive isotopes were pipetted this way in decades past. Although statistical data on lab incidents are very hard to collect and interpret, from the mid-1970s up until the 1990s mouth pipetting was a known cause of lab-acquired infections (*Clin Microbiol Rev*, 8:389-405, 1995). Cases of infections acquired in this way were still occasionally reported in the late 1990s. Mechanical pipettes or automated liquid-handling systems gradually became a lot more accessible and affordable, and now they're fixtures in most life-science labs.

With all the improvements in lab safety, how comfortable do researchers actually feel? A 2013 *Nature* review (493:9-10, 2013) largely based on a survey conducted by the University of California, Los Angeles (UCLA), found that 86 percent of scientists said they felt their labs were safe places to work, but almost half of them had experienced or witnessed a lab accident that resulted in an injury on at least one occasion. Thirty percent of these cases involved a severe injury.

Beyond these surprising statistics, recent years have seen dramatic lab accidents and casualties in both the U.S. and the U.K. The UCLA survey was prompted by the death of a research assistant, who succumbed to burns suffered in a tragic lab fire in the university's Molecular Sciences Building in 2009. Just two years later, a lab accident at Yale University claimed the life of a 22-year old student. And in the U.K. in 2007, foot-and-mouth disease swept through southern England after the virus escaped from the Pirbright Institute for Animal Health. Just two years ago, investigators conducting a routine inventory check at the National Institutes of Health in Bethesda, Maryland, found vials containing live smallpox virus lying abandoned in a general-purpose cold-storage room.

The improvement in lab safety in the last few decades has been largely driven not only by societal and technological change, but also by funding available for the maintenance of lab facilities, training of personnel, and the appointment of dedicated safety officers. Many developing countries are still struggling with the proper upkeep of their research institutions, which consequently affects the safety of those facilities. While in the next 30 years we will surely see a further evolution in laboratory technology and practice, we still have plenty to learn from past and current mistakes.

—Michal Barski

Frankenstein Ants

The cold room in biochemist Danny Reinberg's lab at the New York University Langone School of Medicine is anything but. Illuminated for 12 hours a day and kept at 25 °C, the room is downright balmy. But entomophobes would be well advised to steer clear: this room is filled not with test tubes and petri dishes, but with ants. Lots and lots of ants.

Housed in hundreds of transparent shoebox-size plastic containers, the ants are part of an ongoing effort to establish a new model system for studying behavioral epigenetics, or what Reinberg, who is also a Howard Hughes Medical Institute (HHMI) investigator, calls "epigenetics in action."

The idea of establishing an ant colony as a model system for epigenetics dates back nearly 12 years to a conversation Reinberg had with Shelley Berger, an epigeneticist at the University of Pennsylvania. Berger had recently returned from a family vacation in Costa Rica, where she spent time watching leaf-cutter ants in action. Ant colonies are highly homogeneous, genetically speaking. Yet their members vary dramatically in shape, size, and behavior. "In some cases the worker and queen are absolutely identical genetically, and yet they have completely different functions," Berger explains. "The workers give up their reproduction to the queen." Such phenotypic differences, Berger and Reinberg realized, must come down to epigenetics—variations in gene expression caused by transcription factors, differences in histone modification, noncoding RNA abundance, DNA methylation, and so on.

They recruited ant expert Juergen Liebig of Arizona State University, and the trio settled on a pair of species to study: the carpenter ant *Camponotus floridanus* and *Harpegnathos saltator*, aka Jerdon's jumping ant.

They're still these big soldier-like ants, but now they're foraging. We've changed their brains, sort of the way Dr. Frankenstein changed the behavior of the monster.

—Shelley Berger
University of Pennsylvania

According to Berger, each species offers unique attributes. The carpenter ant has several genetically identical "castes," including a queen and two worker classes with different body morphologies and behaviors: petite foragers and brawny soldiers. The jumping ant has only one worker caste, but unlike workers of most ant species, they retain the ability to become fertile. If the queen dies or is removed from the colony, some of the workers will fight until a winner emerges, at which point she transforms into a queen—a feature that also makes classical genetics studies feasible, as it allows colonies to be manipulated and propagated.

Another benefit of using carpenter and jumping ants as models: a marked difference in life span between castes. A carpenter ant queen may live 10 or 20 years, Berger says, while a forager lasts 6 months. Jumping ant queens live about three times as long as workers, she notes, but "when you do that neat switcheroo trick and make the worker reproductive, the longevity increases"—a difference that is also due to epigenetics.

With $12 million in HHMI funding over eight years, the team has produced

ANTS AND UNCLES: Members of carpenter ant (*Camponotus floridanus*) colonies come in a variety of shapes and sizes that fit into different castes, including the queen (top), major workers (bottom right), minor workers (next to major workers), and reproductive male and female swarmers (bottom left)

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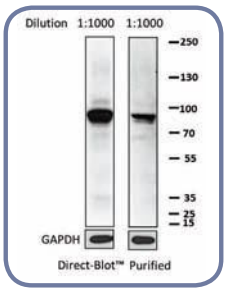

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seven publications, including papers detailing the genomes and methylomes of both species and some of the epigenetic differences that make their transformations tick. Most recently, the team showed that epigenetic manipulation of carpenter ants (using histone deacetylase inhibitors and RNA interference) could turn soldiers into foragers (*Science*, 351:aac6633, 2016). Berger's team calls these creatures "Frankenstein ants," she says, as they blend the behavior of one caste with the morphology of the other: "They're still these big soldier-like ants, but now they're foraging. We've changed their brains, sort of the way Dr. Frankenstein changed the behavior of the monster."

Intriguingly, she says, her lab has found a window early in development when the soldiers are susceptible to such manipulation, after which their predetermined behavioral pattern is locked in. This critical period, Berger adds, is reminiscent of early brain plasticity in humans, and her lab is now working to understand it.

The team initially kept its ants in Arizona, as Liebig already had a working ant facility. Eventually, though, they established colonies in Reinberg's lab in Manhattan, and then in Philadelphia—a process that required a year or so of paperwork and red tape to ensure, among other things, that the animals (one of which is not indigenous to the U.S.) do not escape. Reinberg dedicates multiple technicians to the project, charging them with such tasks as feeding and monitoring the ants, cleaning the room, and performing injections for CRISPR/Cas9-based genetic modification.

Now that the HHMI well has run dry (as of August 31), the team is looking for alternative funding, primarily from the US National Institutes of Health. Berger says she believes the ant queens' extraordinary longevity characteristics will make her research attractive to the National Institute of Aging, while the insects' epigenetically driven behavior could tempt the National Institute of Mental Health.

But nothing is easy when it comes to developing a new model system, Berger notes—especially attracting new converts. The researchers most likely to extend the work are lab alumni, who then take the models to their own newly formed labs. Every trainee, she says, is a "precious emissary."

Roberto Bonasio, Reinberg's former postdoc and now a molecular biologist at the University of Pennsylvania, is one such emissary. As a postdoc, Bonasio led the team's genome and methylome sequencing studies. Now, he plans to study the epigenetic markers of ant behavior. What, Bonasio asks, makes a worker a worker and a queen a queen? "What we think is that there are two softwares in their brains, and one is running and one is paused." In the jumping ant model, he suggests, loss of the queen (and her pheromones) and successful combat with other workers somehow cause those programs to flip, raising a lowly worker to entomological royalty.

Now, Bonasio and his colleagues await the development they hope will elevate ants from curiosity to model organism: genetic malleability. Reinberg says his lab has already developed the tools to propagate and manipulate jumping ants for proper genetic studies, and he hopes to submit a paper to that effect by year's end.

"Those would be the first ant mutants ever made," Bonasio says. "It makes for a movie title: *Ant Mutants in Manhattan!*" Whether it'll be a hit remains to be seen.

—Jeffrey M. Perkel

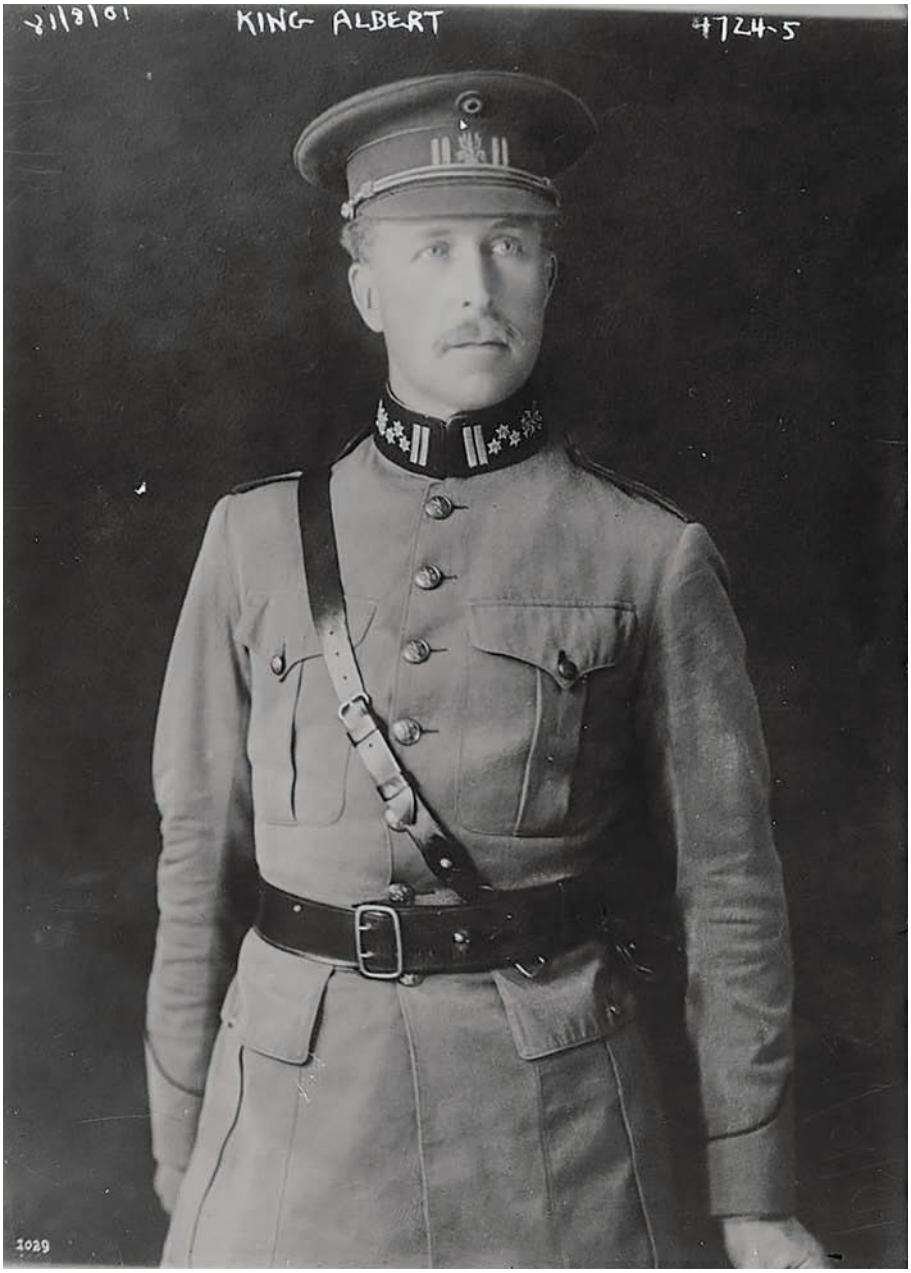
Royal Blood

In 1934, King Albert I of Belgium died in a rock-climbing accident at the age of 58. Eighty years later, Belgian TV journalist Reinout Goddyn purchased some bloodstained leaves supposedly collected from the site of the accident after the body was discovered in the middle of the night. He wanted to settle the conspiracy theories that circulated after the king's death, which no one had witnessed.

In 2014, Goddyn reached out to Dieter Deforce, director of the Laboratory of Pharmaceutical Biotechnology, Forensic DNA, at the University of Ghent, Belgium. "He asked if I could do a DNA analysis to prove if it was the blood of the king or not," recalls Deforce, who was hesitant to help because doing so would require a sample from one of the king's relatives. Given ongoing legal proceedings regarding a woman who claimed to be the daughter of King Albert II, Deforce decided to steer clear of Belgian royalty's genetic data. But he told Goddyn that he could test the bloody leaves to see if the DNA was human or not. Deforce performed genomic and proteomic analyses and demonstrated that the blood from the leaves was from a human. Deforce and Goddyn appeared together on a Flemish television program to discuss the finding later that year.

Watching that broadcast was forensic geneticist Maarten Larmuseau, a postdoc at the University of Leuven, Belgium. But Larmuseau was not satisfied with the results Deforce and Goddyn presented. "That was not the end of the story," he says. "You had to identify with DNA if the blood indeed belonged to the king or not." He reached out to Deforce, who connected him with Goddyn, and the journalist immediately agreed to the project. Larmuseau also contacted two distant relatives of King Albert I—one on the dead king's father's side and one on his mother's side. He planned to compare both Y chromosome and mitochondrial DNA sequences between the living relatives and the blood recovered from the site of King Albert's demise.

The family members agreed, and Larmuseau paid them each a visit to collect saliva samples. He also sampled the blood from Goddyn's leaves. Because the blood was 80 years old, the DNA was highly degraded, but thanks to advances in technologies to deal with such poor-quality samples, Larmuseau and his colleagues were able to run the comparison on 42 Y-chromosome loci and sequence the entire mitochondrial genome. The DNA from the blood was a match with



LONG LIVE THE KING: Conspiracy theories about the death of King Albert I of Belgium are being laid to rest thanks to modern genomic technology.

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both the Y-chromosome loci of his paternal relative and the mitochondrial sequence of his maternal relative (*Forensic Sci Int Genet,* 24:202-10, 2016).

"It could be also the blood of his brother, but he died many years before [King Albert I]," Larmuseau says. "There is only one person who can give this blood."

"It's like a jigsaw puzzle; you're trying to put it together and determine what the picture is," says Michael Baird, chief science officer at DNA Diagnostics Center in Fairfield, Ohio. "The more pieces you have to work with the more likely you are to get the picture and be able to determine what it is. . . . I thought they did a very thorough job in the analysis and were able to identify

with a very high degree of certainty who [the blood] was from."

But the genetic analysis was the easy part, says Larmuseau. Once the researchers had their results, "that's when the problems started." They didn't know how to publish. The raw data contained the Belgian royal family's sensitive genetic information. Comparisons of the Y-chromosome data with other family members' DNA sequences, for example, could identify cases of infidelity, says Larmuseau. Certain mutations on the Y chromosome could also indicate infertility or risk of disease, and publishing mitochondrial sequences carries similar ethical and privacy pitfalls. On the other hand, the researchers couldn't exactly omit the raw data altogether and just ask paper reviewers and the scientific community to take their word for it.

After speaking with other researchers, including several bioethicists, Larmuseau and his colleagues decided to get two independent experts to validate the data and results. Walther Parson of the Institute of Legal Medicine at Innsbruck Medical University in Austria reviewed the mitochondrial data, and Lutz Roewer, a forensic geneticist at the Institute of Legal Medicine and Forensic Sciences at the Charité University Hospital in Berlin, reviewed the Y chromosome analysis. "I totally agree with the authors that such genetic data should not be published and should not be available to the public via the publication," says Roewer. "It's a family case and that's private data. If there is external review that the approach is correct and that the raw data are correct, then it's enough."

> It could be also the blood of his brother, but he died many years before King Albert I. There is only one person who can give this blood.
>
> —Maarten Larmuseau, University of Larmuseau

Larmuseau says that, in addition to personal and historical curiosity, the ethical issues surrounding the publication of historical genetic data is what motivated him to reach out to Deforce and Goddyn in the first place. "For me it was immediately clear that we had to deal with a lot of ethical questions," he says. "It's a sample which is not taken during medical care, so there are no ethical guidelines [regarding the data]. . . . I wanted to make that clear with this sample."

—Jef Akst

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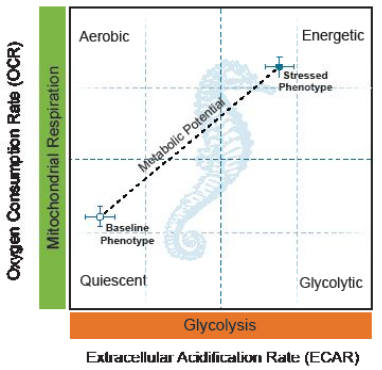

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Cautionary Tale from CRISPR

A patent dispute over gene editing highlights the need for scientists to agree on IP ownership early.

BY CATHERINE COOMBES

The path from the discovery of the CRISPR/Cas mechanism as part of bacteria's adaptive immune system to the use of this pathway in the food industry and later as a breakthrough gene-editing tool is a good illustration of the power of collaboration among scientists across disciplines. Yet CRISPR's development also serves as a salient warning of how failing to assign clear and up-front ownership of intellectual property (IP) can result in prolonged and costly legal friction.

A widely reported patent dispute in the U.S. between the Broad Institute and the University of California, Berkeley, over who first invented the CRISPR/Cas toolkit for gene editing is the subject of interference proceedings now before the Patent Trial and Appeal Board (PTAB) of the US Patent and Trademark Office (USPTO). Less well known is that the earliest CRISPR patent applications filed by the Broad Institute for gene editing are the result of a collaboration among four institutions, and there is an ongoing ownership dispute between Rockefeller University and MIT/Broad over some US CRISPR patents. The fallout from this dispute may have detrimental consequences for Broad's corresponding patents in Europe. Broad's seven granted European CRISPR patents are already embroiled in opposition proceedings before the Opposition Division of the European Patent Office (EPO).

A statement from Broad filed with the USPTO on July 7, 2014, reveals that Broad may not be entitled to rely on the earliest filing dates of December 12, 2012, and January 2, 2013, in Europe. Within 12 months of a first patent application being filed, a subsequent patent application can be filed in another country for the same invention, by the same applicant, and can claim a right of priority to the earlier patent application. This right of priority allows the later-filed patent application to be examined as if it were filed on the same date as the earlier patent application (known as the priority date). This is useful because when the later patent application is examined, if it is entitled to priority, publications that became available between the priority date and the time the patent in question was filed cannot be used in considering whether the application is novel and inventive. However, certain conditions must be met to be able to make this claim.

In Europe, whoever files the later patent application must be the same applicant(s) named on the earlier patent application, or the earlier applicant(s) must have transferred the right to claim priority to the later applicant, who then becomes a "successor in title." This transfer must occur prior to the filing date of the later application.

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MIT/Broad's earliest patent applications, filed at the USPTO in December 2012 and January 2013, named scientists affiliated with four different institutions: Feng Zhang of the Broad Institute and MIT, Shuailiang Lin and Naomi Habib of the Broad Institute, Patrick Hsu and Fei Ann Ran of Harvard College, and Luciano Marraffini of Rockefeller University. It was only after these earliest patent applications were filed that ownership of the inventions arising out of this collaboration was evaluated—and disputed.

According to the statement the Broad submitted to the USPTO, after these priority patent applications were filed in the U.S., the patent attorney who filed the applications undertook an inventorship analysis at the institute's request. The goal of this was to determine which of the named inventors on the US priority documents were entitled to be named as inventors on each of the 10 international Patent Cooperation Treaty (PCT) patent applications that were subsequently filed in December 2013, which claimed a right of priority of these earlier US priority patent applications. Rockefeller University disputed the outcome of these investigations, which resulted in Marraffini not being named as a co-inventor and Rockefeller University not being named as a co-applicant on certain PCT patent applications that related to CRISPR/Cas9 in eukaryotes. Nevertheless, the disputed PCT patent applications were filed by Broad. This

has led to an entitlement dispute between Rockefeller University and Broad in the U.S.

The EPO will not examine ownership, but will look into whether the Broad has a valid right to claim priority. Marraffini was a named inventor and co-applicant on the earliest US patent applications, yet it appears that neither he nor Rockefeller University was an applicant on some of the subsequent PCT patent applications that led to granted European patents. Nor did Marraffini or Rockefeller sign over their priority rights to the Broad. Hence, the formal requirements for claiming priority to these earliest patent applications do not appear to have satisfied European patent law. This could severely limit the scope of the European patents, as highly relevant publications (such as *Science*, 339:819-23, 2013 and *Science*, 339:823-26, 2013) would be citable for novelty and inventive step against these patents. To try to prevent this, MIT/Broad are arguing that the existing case law in Europe should be interpreted to allow the right of priority to be determined based on US law—which does not have the same applicant requirements as European law—because the earliest patent applications were filed in the U.S. For credibility, they have submitted statements by high-profile people in the IP field, such as Lord Leonard Hoffmann (former Lord of Appeal in Ordinary in Britain), John Doll (former commissioner of patents at the USPTO), and Paul Michel (former Chief Judge of the US Court of Appeals for the Federal Circuit).

Unless the Broad successfully convinces the Opposition Division to follow its interpretation of how priority should be determined, these CRISPR patents may provide a high-profile example of the disastrous IP consequences that can occur when collaborations result in disputes. The take-home lesson is that the earlier in the collaboration ownership and management of potential IP rights are addressed, the easier it is to come to agreement between the parties. Before entering into a collaboration consider why you are seeking to collaborate. What is each party bringing to the table? Can ownership of any potential IP be considered from the outset?

Various toolkits can aid parties in deciding ownership of IP within a collaboration, such as the Lambert toolkit, UK-Korea collaborative research IP toolkit, UK-India collaborative research IP toolkit, and European Commission Cross Border Decision Guide. However, a collaborative agreement can be any written document agreed to by all the collaborators. The technology transfer offices of your institution will also be able to help.

In addition to seeking external support, scientists should maintain a record of what contributions are made by whom and keep copies of all correspondence. Looking at lab books retrospectively can indicate who carried out the experiments, but it may be unclear who devised the experiments that were conducted. Keeping a paper trail is key. When scientific accolades and vast commercial potential are at stake, it is understandable how disputes can arise. ■

Catherine Coombes is a senior patent attorney with HGF Limited in the U.K. This article first appeared on the-scientist.com August 31, 2016.

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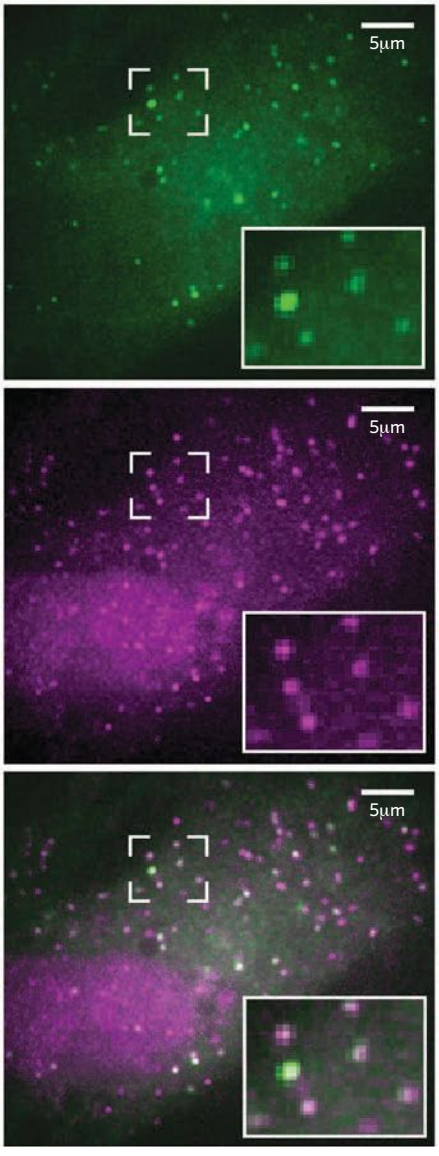
Live-Action Translation

Four independent research groups develop techniques for visualizing peptide production in living cells.

BY RUTH WILLIAMS



Sixty years ago, Francis Crick proposed the central dogma of cell biology: DNA makes RNA makes protein. Back then, watching that sequential process in actual living cells no doubt seemed the stuff of science fiction.

But by 1996, researchers had figured out a way to view individual DNA loci in live cells. They engineered the cells' genomes to contain tandem repeats of a specific nucleotide sequence, introduced green fluorescent proteins that would bind to the repeats, and observed the resulting cluster of fluorescent proteins—which appeared as a bright spot in the nucleus—under the microscope.

Shortly afterwards, scientists came up with a similar trick for visualizing single mRNA molecules: fluorescent antibodies were targeted to a string of repeated stem-loops, RNA sequences designed to fold back on themselves. And by 2004, a few months before Crick's death, it was possible to combine these two techniques and watch transcription in real time.

"You had an RNA being born from a particular locus of DNA—we could see where that was, and we could track the RNA out [of the nucleus]," says Tim Stasevich of Colorado State University. But synthesis of the protein itself was still unobservable, he adds. "You could say, it got lost in translation."

NEWBORN PROTEINS: Researchers can spot translation in progress by the combined presence of fluorescent tags that stick to newly formed peptides (green) and their corresponding mRNA (pink).

More than a decade later, Stasevich and colleagues, alongside three independent groups, have succeeded in devising techniques for visualizing single-molecule translation in living human cells. "The fact that you have four labs working on this is a testament to how hot the topic is," he says.

Stasevich's group engineered expression vectors that produced an mRNA containing stem-loop epitopes and encoding proteins in which the first few hundred amino acids formed a peptide domain of repeated epitopes called FLAG-tags. This domain, which the team dubbed "the spaghetti monster," binds multiple FLAG-specific fluorescent antibodies that are injected into the cell, while the mRNA binds its own fluorescent antibodies. Thus, both the mRNA and its newly forming protein are observable at once (*Science*, 352:1425-29, 2016).

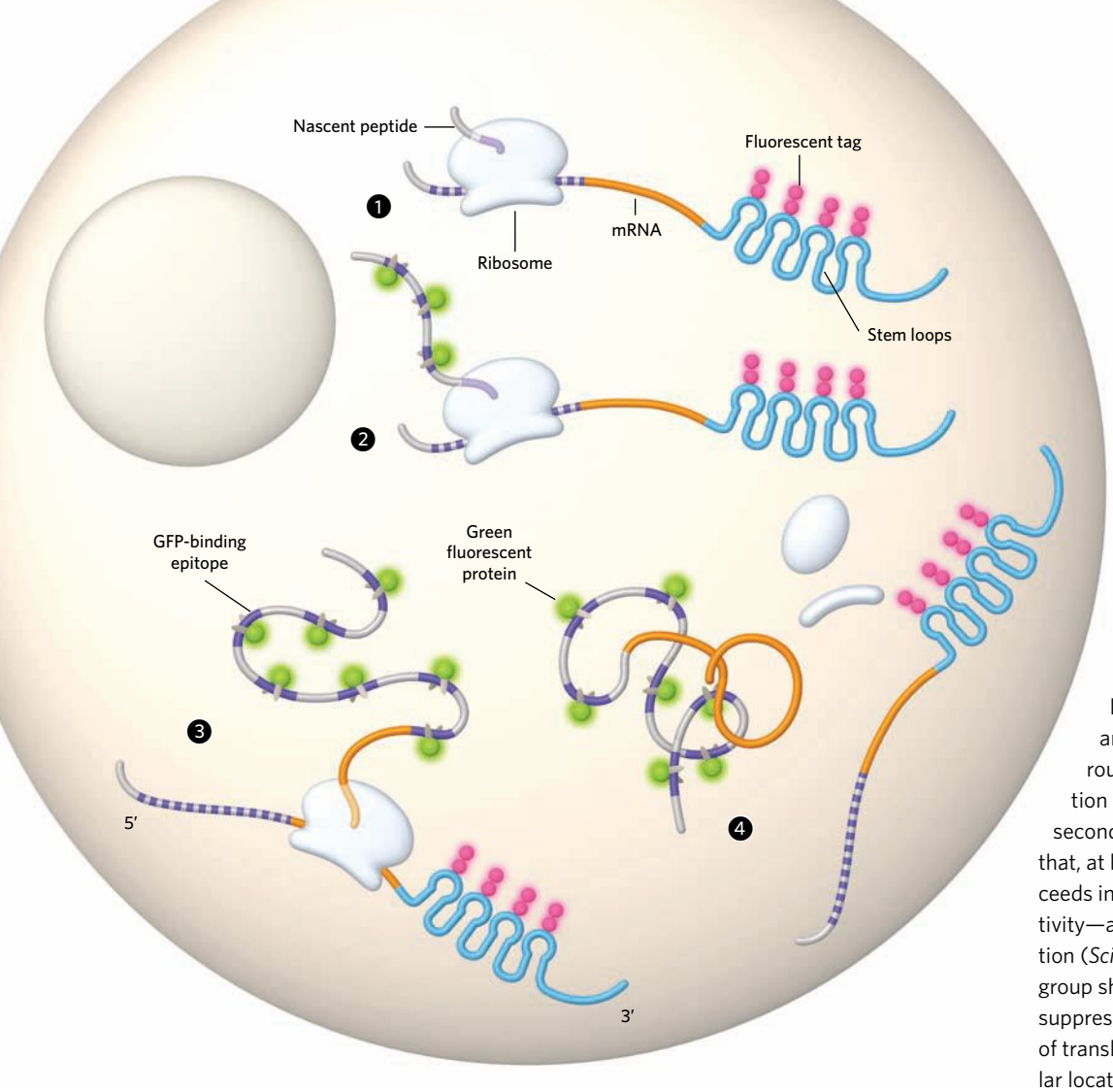
The techniques of the other three groups—led by Robert Singer of Albert Einstein College of Medicine, Marvin Tanenbaum of the Hubrecht Institute in the Netherlands, and Xiaowei Zhuang of Harvard University—used either the same or similar stem-loop epitopes for mRNA detection as Stasevich, but made use of a system called SunTag, developed by Tanenbaum in 2014, for detecting the proteins' translation. SunTag, like the spaghetti monster, is an array of epitopes that bind fluorescent antibodies, but SunTag antibodies are encoded in a separate genetic construct, rather than injected into the cell.

Introducing antibodies into a cell "can be challenging," admits Stasevich, "so I think

AT A GLANCE

TRANSLATION TRACKER	PROTEIN DETECTION	RNA DETECTION
SunTag	24 SunTags (each 19 amino acids in length, separated by linkers of five amino acids) bind fluorescent, single-chain variable fragment antibodies.	Fluorescent MS2 coat protein (MCP) or PP7 coat protein (PCP) recognizes stem-loop structures encoded in mRNA.
Spaghetti monster	10 FLAG-tags (8 amino acids each) or 10 HA-tags (9 amino acids each), interspersed within a peptide domain bind fluorescent antibody fragments.	Fluorescent MCP recognizes MS2 stem loops.

CELL, 165:990-1001, 2016



TRANSLATION ORIENTATION: Four independent teams of researchers have designed methods to observe translation in living cells, all based on the same basic approach. They engineered an mRNA to fold repeatedly into so-called stem loops in its 3′ untranslated region (blue). A fluorescent protein (pink) binds to these loops to allow for mRNA tracking. To observe the newly formed peptide, the researchers built in repeated peptide sequences (purple) that bind green fluorescent protein (green).

that's definitely one of the advantages of the SunTag [method]." However, a benefit of injecting rather than encoding antibodies, he says, is that it's easier to control the amount of antibody—and, therefore, to get a good signal with little background. In addition, Stasevich's system allows researchers to "image the proteins not just in one color but two," he says. Indeed, by encoding a different spaghetti monster domain (containing epitopes called HA-tags) in a second mRNA, the team observed two different translation events in the same cell.

Aside from a handful of minor pros and cons, the techniques all work on a similar principle, and, importantly, "they provide insights and answers to questions that wouldn't have been possible by biochemical means," says translation researcher Nahum Sonenberg of McGill University who was not involved in any of the studies. The ability to look at individual mRNA dynamics is a "big advance," he adds.

The groups examined the translation kinetics for individual mRNAs and found that translation initiated roughly twice a minute and that elongation proceeded at 3 to 10 amino acids per second. Singer's crew also found evidence that, at least in neurons, translation proceeds in bursts of activity followed by inactivity—as has been described for transcription (*Science*, 352:1430-35, 2016). Zhuang's group showed how environmental stresses suppress translation and how the dynamics of translation vary depending on subcellular location (*Cell*, 165:990-1001, 2016). And Tanenbaum's team observed that individual mRNAs from the same gene in the same cell can vary dramatically in their translation efficiency, with some being practically silent and others translating robustly (*Cell*, 165:976-89, 2016). "You wouldn't have appreciated that heterogeneity if it weren't for these single-molecule techniques," says Sonenberg.

In addition to revealing the spatial and temporal dynamics of translation, the new techniques, if combined with the visualization of specific genetic loci, could even allow researchers "to see the entire central dogma playing out in a cell," says Tanenbaum. "And that would be pretty cool." ■

GENETICALLY ENCODED?	POSSIBLE TO DETECT TRANSLATION OF MULTIPLE RNAS?
Fully	No. Theoretically, could be combined with spaghetti monster system
Partially: mRNA is encoded in a plasmid, but anti-FLAG and anti-HA antibody fragments must be transduced into cells	Yes. Possible to detect two different RNAs and their respective proteins

Thirty Years of Progress

Since *The Scientist* published its first issue in October 1986, life-science research has transformed from a manual and often tedious task to a high-tech, largely automated process of unprecedented efficiency.

It's easy to take for granted the widespread use of optogenetics, CRISPR, and direct cell reprogramming. But there was a once a time when these techniques were impossible—even unimaginable. In celebration of the magazine's 30th anniversary, we are taking a look back at five fields that have embraced technological sea changes, enjoying precise and powerful methodologies and instrumentation that have enabled revolutionary biological insights. Here we reflect on the pioneers whose innovations have propelled advances in microscopy, sequencing, brain imaging, gene editing, and stem cells.

30 years of DNA sequencing

University of Oklahoma graduate student Richard Wilson spent the early 1980s reading DNA. First he'd add four radioactively labeled synthesis-terminating nucleotides—one corresponding to each of the four natural bases—to mixtures of DNA fragments. He'd then load fragments treated with different radioactive bases into separate wells of a polyacrylamide gel and use electrophoresis to separate the strands into a pattern that reflected their length, and, consequently, where the unnatural bases had incorporated.

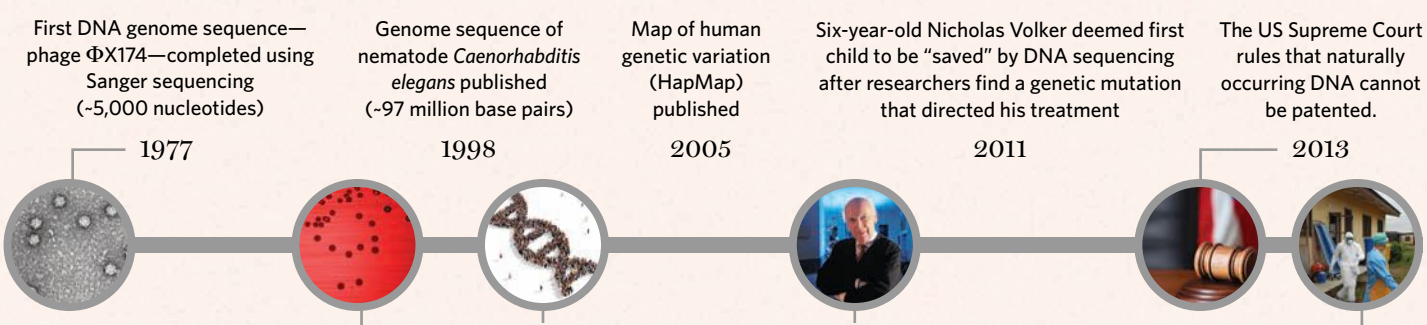
"It was all very manual," recalls Wilson, now director of the McDonnell Genome Institute at Washington University in St. Louis. "We used to get the sequencing gels running, go have dinner and probably a few beers. Then we'd come back to the lab around two in the morning, take the gels down, put X-ray film on them, and develop them the next day."

Wilson and his lab mates would gather in an office and read out the order of bases from the X-ray films, going from shortest fragment to longest fragment, as someone typed the sequence into a computer. In this way, running four electrophoresis experiments at once—"or eight if we were feeling adventurous," Wilson says—the team completed the 17,553-base mitochondrial genome of the frog *Xenopus laevis* in about two years (*J Biol Chem*, 260:9759-74, 1985).

The method, known as dideoxy or Sanger sequencing, was published in 1977 and was one of the first widely adopted techniques for reading DNA. For a decade, it was carried out manually in labs around the world, and used mainly to sequence individual genes and small viral genomes. But things were about to change. DNA sequencing was on the cusp of a technological revolution that would kick-start a succession of ever-faster, cheaper, and more-accurate methods, shifting the field of genomics into the high-throughput powerhouse of scientific data generation that it is today.

"It was clear to us that automating DNA sequencing was really going to be

MILESTONES



1977: SHIFTFN/WIKIMEDIA COMMONS; 1995: CDC/DR. W.A. CLARK (PHIL #1617), 1977; 2001: ARTHIMEDES/SHUTTERSTOCK.COM; 2007: COLD SPRING HARBOR LABORATORY; 2013: ALEX STAROSELTSEV/SHUTTERSTOCK.COM; 2016: CDC GLOBAL

key to the future of biology," says Leroy Hood, who cofounded Applied Biosystems Inc. (ABI) in 1981 to develop some of the instruments that would drive this revolution. "Molecular biology was coming to the fore, and it was clearly central to understanding biological information in living organisms. . . . Sequencing was going to become very, very important."

Ramping up

In 1986, ABI announced the first automated DNA sequencer. Although based on the Sanger technique, the new machine used fluorescent, not radioactive, labels. With one color for each nucleotide, that meant sequencing one section of DNA required just one lane in a gel instead of four (*Nature,* 321:674-79). After electrophoresis, the base sequence could be read from the gel by a computer equipped with a lens and photomultiplier. Later versions of the technology incorporated automatic lane loading, too.

It wasn't cheap, costing between $2 and $5 per sequenced base, but reading DNA suddenly became more practical. In 1990, Wilson, along with a team of researchers at Washington University in St. Louis and the Laboratory of Molecular Biology in Cambridge, set out into uncharted territory—sequencing the whole nuclear genome of a multicellular animal, the nematode *Caenorhabditis elegans*—using ABI machines. After eight years, the 97-megabase project was deemed complete. Meanwhile, also starting in 1990, a much larger international team was tackling an even bigger project: the sequencing of the 3.3 billion nucleotides making up the human genome.

"We thought it would be transformative," says Kim Worley, a geneticist at Baylor College of Medicine who was involved in the Human Genome Project. "Every lab around the world was spending lots of time analyzing one part of one gene. Giving people all the genes, all at once, so they could just do the biology would be a tremendous benefit." Ten years and $3 billion later, the Project's members completed a draft of the human genome.

Working in parallel

As researchers sifted through the data pouring out of these projects, a wave of technologies that would become next-generation (next-gen) sequencing was already gathering steam. These technologies used massive parallelization, with the ability to produce "millions of different sequences simultaneously, but with very short reads," Hood says.

In the first commercially successful next-gen sequencers, released by 454 Life Sciences in 2005, parallelization was achieved via rapid amplification of small, bead-bound fragments of DNA using polymerase chain reaction (PCR). And nucleotides were read using a technique called pyrosequencing (*Nature,* 437:376-80, 2005). The system could sequence 25 million bases with 99 percent accuracy in a single 4-hour run—a 100-fold increase in throughput—at less than one-sixth the cost of conventional methods.

The following year, Solexa (acquired by biotech giant Illumina in 2007) presented its take on next-gen sequencing, introducing the technology that is most widely used today. Instead of bead-based amplification, Illumina machines employ a technique called bridge amplification to clone fragments of single-stranded DNA immobilized in a flow cell (*Nature,* 456:53-59, 2008). The sequences themselves are read using fluorescently labeled nucleotides similar to those of the Sanger method. Along with their offshoots, these technologies have come to dominate research and clinical labs as the cheap and effective sequencers of choice; the release of Illumina's HiSeq X Ten system in 2014 brought the cost of sequencing a human genome below the $1,000 mark.

"Now, my students, some of whom don't know any sequencing, think nothing of it," says Harvard University's George Church, who pioneered one of the first next-gen bead-based methods back in 2005 (*Science,* 309:1728-32). "If they change one base pair in the human genome, they'll send it out for sequencing and check they changed that base pair and nothing else. That's kind of a

TECHNOLOGIES

Walter Gilbert (top), working with Allan Maxam, and Frederick Sanger (bottom) independently publish their sequencing methods.

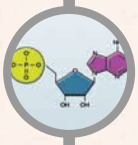
1977

US biochemist Kary Mullis introduces polymerase chain reaction (PCR) to copy DNA.

1985

Applied Biosystems launches four-color fluorescence automated DNA sequencer based on the Sanger method.

1986



Researchers publish pyrosequencing method, based on detecting release of pyrophosphate during DNA synthesis.

1996

454 Life Sciences launches first next-generation sequencer, based on bead-bound DNA amplification and the pyrosequencing technique.

2005

Solexa launches a next-generation sequencer, the Genome Analyzer, building on a technique similar to Sanger sequencing.

2006

Pacific Biosciences commercializes single-molecule real-time (SMRT) sequencing.

2011

Oxford Nanopore Technologies announces MinION, a single-molecule sequencer the size of a USB memory stick.

2012

Illumina releases HiSeq X Ten, which achieves the first $1,000 human genome.

2014

1977: SANGER—NIH, GILBERT—NATIONAL LIBRARY OF MEDICINE; 1996: *TS* STAFF; 2005: COURTESY OF ROCHE; 2006: *BIOCHEMICAL SOCIETY TRANSACTIONS* FEB 01, 2015,43(1):1-5; DOI: 10.1042/BST20140254; 2014: COURTESY OF ILLUMINA

ridiculous assay by 1980s standards, but it actually makes sense today."

New frontiers

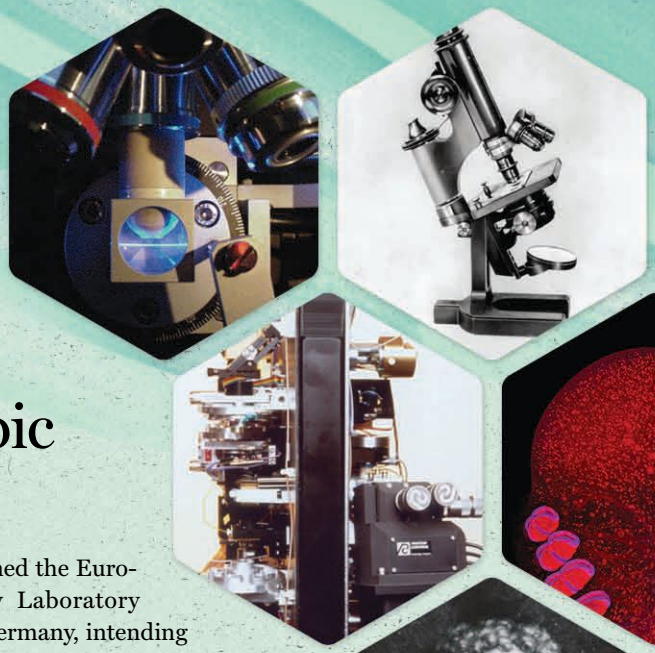
The sequencing field shows no signs of slowing down. Today, emerging technologies such as single-molecule real-time (SMRT) and nanopore sequencing are beginning to eliminate the need for amplification, with advantages that go beyond just increasing speed: in addition to reducing PCR-derived bias and permitting longer reads, these single-molecule techniques retain DNA-bound molecules so researchers "could read out methylation and footprinting information," Church notes, presenting the possibility of obtaining genetic and epigenetic reads simultaneously. (See "Sons of Next Gen," *The Scientist,* June 2012.)

Such "third-generation sequencing" is already making its debut in biomedical research. Earlier this year, for example, scientists used Oxford Nanopore's portable MinION device to classify Ebola strains in West Africa with a genome sequencing time of under 60 minutes (*Nature,* 530:228-32). The same device is currently being used in Brazil to map the spread of Zika virus across the country in real time, and was used this summer to sequence DNA on the space station.

Of course, these nascent technologies are not without problems, says Wilson. "I would say there's not much that's really shown itself to be incredibly robust," he notes. "If you're going to use those technologies, either in the research or clinical setting, you've got to be able to get consistent results from experiment to experiment. I'm not sure we're quite there yet."

But according to Hood, now 77 years old and president of the Institute for Systems Biology in Seattle, that transition is just on the horizon, and will reinforce the remarkably swift scientific progress that has characterized the last 30 years of DNA sequencing. "Living through it, you were very impatient, and always wondered when we'd be able to move to the next stage," he reflects. "But in looking back, all of the things that have happened since '85, they're really pretty astounding."

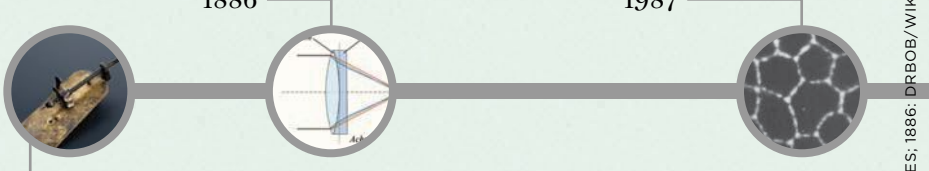
—Catherine Offord



Microscopic Magic

In 1983, Ernst Stelzer joined the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, intending to do his doctoral research on membrane protein dynamics. Soon, he picked up a side project: developing one of the first confocal fluorescent microscopes. Stelzer and his colleagues designed a device with a pinhole that filtered out-of-focus layers of laser light to create thin "optical sections"—as opposed to physical slices—of a thick sample (*J Microsc,* 138:29-34, 1985). It was the first time anyone had been able to image intact tissue in three dimensions.

Stelzer recalls taking an entire day to capture four images of baby hamster kidney cells. In addition to prepping the sample and focusing the microscope over the tissue, he had to transfer the resulting images from the microscope's internal memory to floppy disk to scanner and, finally, to film printer. But despite its labor-intensive nature, the homemade instrument soon became a workhorse of the lab. Stelzer and his collaborators spent the next several years imaging subcellular structures and processes, such as the organization of microtubules, the synthesis and transport of lipids in canine kidney cells, and the tight junctions that join bird gizzard muscle cells.



1673

Antonie van Leeuwenhoek reports his first findings using single-lens microscopes, which resolved structures as small as 1 μm in size. (The first microscope was reportedly built in 1595, but observations made with the instrument were not reported.)

1886

Ernst Karl Abbe, Carl Zeiss, and Otto Schott introduce the apochromat lens microscope, achieving Abbe's diffraction limit (around 200 nm, a fivefold improvement on van Leeuwenhoek's instrument).

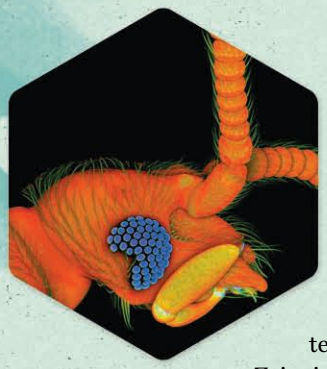
Around 1980

Multiple technologies converge to make higher-resolution microscopy possible: commercially available lasers, better semiconductors for more-sensitive detectors, more versatile fluorescent dyes, and improved computing power for image processing.

1987

Groups at EMBL in Heidelberg and the Medical Research Council in Cambridge, U.K., use newly developed confocal microscopy to observe lipid transport and cellular structures, such as mitotic spindles and the endoplasmic reticulum, in unprecedented detail.

1673: WELLCOME IMAGES; 1886: DRBOB/WIKIMEDIA COMMONS; 1987: ERNST STELZER



Stelzer soon began working with the Zeiss company to build some of the first commercial confocal instruments, and by the mid-1990s, the technique began to be widely adopted, revealing 3-D structures that traditional light microscopy never could. And confocal microscopy was just the beginning of the imaging revolution, which ultimately led to today's ultra-high-resolution techniques and greatly improved three-dimensional visualization of living specimens. Now, these methods are drastically reshaping biologists' ability to see and understand life's smallest details—and the tool kit continues to grow.

Chasing physical limits

The first microscopes were developed in 17th-century Holland, with notable contributions from Dutch lens maker Antonie van Leeuwenhoek, who built instruments with resolutions as good as 1 micrometer (μm). In 1873, physicist Ernst Karl Abbe figured out that the resolution of a microscope was limited by the diffraction of the light passing through its lens, and worked with optical instrument maker Carl Zeiss (founder of the Zeiss company) and glass manufacturer Otto Schott to develop microscopes capable of resolving images right up to what he proposed as the theoretical limit—about 200 nanometers (nm), or 0.2 μm. Abbe argued that it would not be possible to achieve finer resolution, and for the next century, that seemed to be the case. "The microscope you could buy in 1980 wasn't terribly different from the microscope you could buy from Zeiss in 1880," says Eric Betzig of the Howard Hughes Medical Institute's Janelia Research Campus. Yet today, researchers can buy microscopes with resolution 10 times greater than Abbe's diffraction limit.

When Betzig entered graduate school in 1982, he built a microscope with 12-nm resolution by passing light through holes smaller than the light's wavelength. The instrument rapidly lost focus when positioned more than 20 nm from the sample, however; Betzig needed another way into biological samples. Meanwhile, Stefan Hell—first as a graduate student at the University of Heidelberg, then as Stelzer's postdoc at EMBL, and, finally, in his own group at the Max Planck Institute for Biophysical Chemistry—was also trying to break the diffraction barrier. His idea was to add more energy to an already excited fluorescent molecule to force it to drop to a lower energy state, effectively reducing the background signal by quenching the fluorescence of objects outside of the desired area. "In those days, scientists believed that it would be unfeasible," Hell recalled in an email. "I wanted to know if it was possible."

Hell's group succeeded first. In 2000, the researchers introduced a high-energy method known as stimulated emission depletion (STED). The microscope projects a narrow beam to excite a small part of the sample while simultaneously shining a ring around the narrow beam to quench the surrounding fluorescence, shrinking the area that is illuminated. It was the first optical method to significantly push past the diffraction limit for biological specimens, revealing clear delineations between structures as close as 20 nanometers apart (*PNAS*, 97:8206-10, 2000).

1994

Green fluorescent protein (GFP) is used for the first time as a protein marker in living cells, revolutionizing biologists' ability to image live cells using fluorescent tags.

2000

Stefan Hell develops stimulated emission depletion (STED) technology, the first optical technique to surpass Abbe's diffraction limit in biological samples.

2000

Mats Gustafsson is among the first to introduce structured illumination microscopy (SIM), which shines patterns of light on a sample to reduce the amount of energy directed at the sample while boosting resolution.

2004

Ernst Stelzer unveils selective plane illumination microscopy (SPIM), a method that drastically reduces the light energy directed at a sample by shining it in sheets perpendicular to the detector, and makes it possible to image animal and plant embryos for more than 100 hours without damaging them.

2006

Eric Betzig finds a new way to break the diffraction barrier with photoactivatable fluorescent molecules first identified by William Moerner, developing photoactivated localization microscopy (PALM).

2014

Shortly after Hell, Moerner, and Betzig are announced as winners of the 2014 Nobel Prize in Chemistry, Betzig publishes a description of a new technique that melds the advantages of SPIM and SIM: lattice light-sheet microscopy.

2000: ERNST STELZER; 2004: PHILIPPE GIRARD; 2006: ZEISS MICROSCOPY; 2014: BETZIG LAB, HHMI

STED "radically changed people's mind-set about what a light microscope can actually do," Hell says.

Six years later, Betzig, too, built a microscope with resolution greater than Abbe's 200-nanometer diffraction limit. To do so, he had turned to William Moerner's discovery of a GFP variant that could be turned on and off with different wavelengths of light. Betzig's method, called photoactivatable localization microscopy (PALM), uses slight differences in the on/off rates of individual molecules to distinguish between them and determine their exact location from a series of superimposed images; the method has a resolution comparable to that of STED (*Science,* 313:1642-45, 2006). He, Moerner, and Hell shared the 2014 Nobel Prize in Chemistry for their advances in super-resolution microscopy, or nanoscopy.

The microscope you could buy in 1980 wasn't terribly different from the microscope you could buy from Zeiss in 1880.

—Eric Betzig, Howard Hughes Medical Institute

Sheets of light

One drawback of super-resolution techniques is potential damage to the sample being examined from the extreme amount of energy directed at it. "STED requires a billion times more light than cells evolved [to live] at," Betzig says. Hell has partially solved this issue with RESOLFT, a STED variation that uses much lower levels of light. Scientists have also developed other "gentler" methods, such as structured illumination microscopy (SIM), which shines patterns of light on a sample. The resulting images can then be combined for a twofold improvement in resolution over Abbe's limit, and SIM "doesn't locally burn your sample like a confocal technique," says Rainer Heintzmann of the University of Jena who began developing the method as a graduate student in the late 1990s.

But even these lower-energy confocal techniques can damage cells. For imaging live tissue in three dimensions over long periods of time, researchers turn to light-sheet microscopy, in which light shines through the side of the sample perpendicular to the detector. By 2004, Stelzer had developed selective plane illumination microscopy (SPIM), a technique that achieved subcellular resolution, and was using it to visualize live embryos of fish and flies (*Science,* 305:1007-09, 2004). Because SPIM exposes samples to energy that is several orders of magnitude less intense than other techniques, imaged samples go on to produce viable offspring, Stelzer says. Most recently, Betzig introduced lattice light-sheet microscopy, which reduces the light energy directed at the sample by melding SPIM with SIM, illuminating the specimen with patterns of light instead of flat sheets (*Science,* 346:1257998, 2014).

"People really do experiments that simply were not possible before," says Stelzer. **—Jenny Rood**

Untangling the Brain

In the mid-1980s, György Buzsáki was trying to get inside rats' heads. Working at the University of California, San Diego, he would anesthetize each animal with ether and hypothermia, cut through its scalp, and drill holes in its skull. Carefully, he'd screw 16 gold-plated stainless steel electrodes into the rat's brain. When he was done with the surgery, these tiny pieces of metal—just 0.5 mm in diameter—allowed him to measure voltage changes from individual neurons deep in the brain's folds, all while the rodent was awake and moving around. He could listen to the cells fire action potentials as the animal explored its environment, learning and remembering what it encountered (*J Neurosci,* 8:4007-26, 1988).

In those days, recording from two cells simultaneously was the norm. The 16-site recording in Buzsáki's 1988 study "was the largest ever in a rat," he says. Nowadays, scientists can measure voltage changes from 1,000 neurons at the same time with silicon multielectrode arrays. But the basic techniques of using a probe to measure electrical activity within the brain (electrophysiology) or from outside it (electroencephalography, or EEG) are still workhorses of neural imaging labs. "The new tools don't replace the old ones," says Jessica Cardin, a neuroscientist at the Yale School of Medicine. "They add new layers of information."

Another decades-old neuroscientific technique that remains popular today is patch clamping. Developed in the late 1970s and early 1980s, it can detect changes in the electric potential of individual cells, or even single ion channels. With a tiny glass pipette suctioned against the cell's membrane, researchers can make a small tear, sealed by the pipette tip, and detect voltage changes inside the cell. With some improvements, the patch clamp, like electrophysiology and EEG, has remained a regular part of the neuroscientist's tool kit. Recently, researchers had a robot carry out the process (*Nat Methods*, 9:585-87, 2012).

However, patch clamping is invasive, records only from single cells, and can't measure over long periods because the process can interfere with the cell's normal functioning. Instead, some scientists use a less invasive, more durable, and higher-throughput approach first described half a century ago: calcium imaging (*J*

1924: ANDRII CHERNINSKYI/WIKIMEDIA COMMONS; 1976: N. FERTIG/WIKIMEDIA COMMONS; 1980's: RADIOLOGY, UPPSALA UNIVERSITY HOSPITAL; 2001: AKERBOOM, RIVERA, GUILBE, MALAVÉ, HERNANDEZ, TIAN, HIRES, MARVIN, LOOGER, SCHREITER ER/WIKIMEDIA COMMONS

2005: MIT MCGOVERN INSTITUTE, JULIE PRYOR, CHARLES JENNINGS, SPUTNIK ANIMATION, ED BOYDEN; 2007: JEFF W. LICHTMAN AND JOSHUA R. SANES/WIKIMEDIA COMMONS; 2015: ED BOYDEN, FEI CHEN, PAUL TILLBERG/MIT

Cell Comp Physiol, 59:223-39, 1962). When an action potential reaches an axon terminal, calcium ions rush inward. To detect when this happens, scientists use molecules that fluoresce when they bind calcium.

Initial efforts were clunky, however, and the technique's popularity surged only after calcium sensors and microscopy techniques improved in the 1990s and early 2000s. Now, one of the most popular sensors is the genetically encoded calcium indicator (GECI) GCaMP6, a fusion of green fluorescent protein and the calcium-binding protein calmodulin (*Nature*, 499:295–300, 2013). The proteins glow like lightning bugs when a neuron fires. By thinning an area of an animal's skull to create a "cranial window," scientists can watch when and where action potentials occur in the outer layers of the cortex. "You can take a mouse, alive and functional, with the head fixed in one spot. But the animal still can run on a treadmill," says David Prince, a neurologist at the Stanford University School of Medicine. "You can detect fluorescent signals in his brain . . . and try to relate those changes to changes in his behavior that are simultaneously occurring."

But calcium imaging has its own drawbacks. It can only measure changes in electrical activity on 50- to 100-millisecond time scales, while an action potential occurs in just 1 millisecond. Patch clamping and other electrodes, on the other hand, can measure individual action potentials.

To complement ongoing advances in eavesdropping on the brain's neural chatter, scientists have developed new techniques for visualizing the brain's structure. For example, last year, MIT synthetic neurobiologist Ed Boyden and his colleagues introduced expansion microscopy, which allows a brain tissue sample to be expanded to as much as 100 times its original volume while preserving the arrangement of the molecules. With the addition of vibrant color probes, the team imaged nanoscale features of cells and synapses in the mouse hippocampus (*Science*, 347:543-48, 2015). Understanding the anatomy of the brain better will help reveal how it works, Boyden says. "Anatomy's the kind of thing where if you have enough information about something, it actually gives you function."

1801

Transcranial direct current stimulation (tDCS) first used on humans: A small current applied to the scalp stimulates brain regions locally.

1875

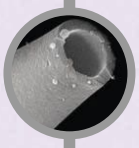
Electroencephalography (EEG) first used: Electrodes placed on the brain surface of experimental animals record regions of neural activity.

1924

EEG first performed on humans

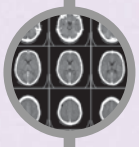
1940

First recordings of brain cells with microelectrodes, performed in cats and rabbits



1976

Patch clamp invented: A glass pipette affixed to a neuron cell body measures changes in voltage potential.



mid-1980s

Micrometers-thin slices of brain tissue kept alive in chambers and infused with drugs or neurotransmitters allow for recording electrical responses of neurons from known areas of the brain.

1985

Transcranial magnetic stimulation (TMS) invented: A noninvasive manipulation of the brain by repeated pulses of a magnetic field

2001

Genetically encoded calcium indicator (GECI) called GCaMP invented: Transgenic protein in animal models glows when it binds calcium ions associated with action potentials.

2005

Optogenetics used to activate and inhibit mammalian neurons with light ex vivo

2007

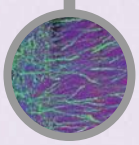
Designer receptors exclusively activated by designer drugs (DREADDs) invented: A method for manipulating neurons with a non-natural receptor and synthetic drug pair

2007

Brainbow developed: The random expression of green, red, and blue fluorescent proteins combines into about 100 unique colors to clearly distinguish individual neurons.

2013

CLARITY invented: A technique to make tissues transparent using acrylamide hydrogels that assemble in situ



2015

Expansion microscopy invented: A technique for expanding tissue samples while keeping the organization of molecular structures intact

Brain control

Manipulating the brain can also shed light on how it works. In the late 1960s, Jose Delgado of Yale University placed electrodes in the brain of a chimpanzee named Paddy to alter the animal's emotional behavior. The transmitter produced an unpleasant sensation in response to a specific pattern of activity in Paddy's amygdala. After six days of repeated stimulation, she grew depressed, and the activity pattern decreased by 99 percent. Paddy recovered after two weeks, but when Delgado repeated the experiment, she became melancholic again. "What he did was incredible," says Buzsáki, now at New York University, because Delgado could listen to and precisely manipulate brain waves in an era when vacuum tubes were considered high tech.

MENTAL MAPS

Even though researchers now have tools to record from hundreds of neurons simultaneously and to manipulate small populations of cells in rodent brains, the human brain remains largely a mystery. With some 86 billion neurons linked by 100 trillion synapses, the three-pound organ remains a scientific puzzle of epic proportions.

Magnetic resonance imaging (MRI), used for the first time on a human in 1977, enabled researchers to noninvasively image the structure of a person's brain. For taking pictures of the brain, MRI was easier to perform than its predecessor, positron emission tomography (PET), which required intravenously injecting radionuclide-labeled metabolites to assess brain activity. Marcus Raichle, a neurologist at Washington University in St. Louis who helped develop PET in the 1970s, recalls his reaction: "My God, if you had an MR[I] scanner, you were in the brain-mapping business."

But what catapulted MRI into a mainstay of neural imaging was the discovery of functional MRI (fMRI) scanning. In 1990, Seiji Ogawa, then at AT&T Bell Laboratories, and colleagues showed that deoxygenated blood responds differently to a magnetic field, and that this could be used as an internal contrast agent to illuminate changes in the brain (*PNAS,* 87:9868-72, 1990). As a subject in an fMRI scanner performs a simple task, researchers can use the increased flow of oxygenated blood in different regions of the brain as a proxy for neural activity.

Another variant of MRI has also made waves: diffusion MRI. This technique traces the axons of neurons by the Brownian motion of water molecules, which are more likely to diffuse along the axon's fibrous structure rather than perpendicular to it. The method traces the trajectories of fibers between gray matter regions, dense with neuronal cell bodies. The National Institutes of Health–funded Human Connectome Project is using this tool and those above to map all the connections in the brain—a figure greater than the number of stars in the sky.

Over the past 50 years, with ever more compact and precise electrodes, researchers have continued to use electromagnetic stimulation to manipulate neural activity with the goal of understanding brain function. Transcranial magnetic stimulation, or applying a noninvasive magnetic field outside the head, has been used to reveal the physiological underpinnings of neurological diseases and identify potential treatments in animal models, for example. And following in Delgado's footsteps, Buzsáki and his colleagues have obliterated ripples of neural activity responsible for consolidating the day's memories in sleeping rats (*Nat Neurosci*, 12:1222-23, 2009). "The animal remembers nothing the next day, even though it had perfect sleep," he says.

In 2005, a powerful alternative to electrodes and magnetic fields emerged: controlling neurons with light. Boyden, along with Stanford's Karl Deisseroth and their colleagues, inserted algal ion channels that respond to light into mammalian neural cells. With photons, they could depolarize a cultured neuron's membrane at will (*Nat Neurosci,* 8:1263-68, 2005), and researchers soon used the tool in vivo. Named science's Method of the Year by *Nature* in 2010, optogenetics allows researchers to target specific cell populations. The technique has been used in mice to alter (*Science,* 341:387-91, 2013) or trigger a memory (*Nature,* 484:381-85, 2012), shut down epileptic seizures (*Nat Commun,* 4:1376, 2013), and inhibit aggressive behavior (*Nature,* 470:221-26, 2011).

Another technique relies on chemistry, rather than light. Scientists deliver G-coupled receptors, created through directed evolution, to cells in an animal's brain. Then, by injecting a synthetic ligand into the body, scientists can trigger neuron firing or silencing (*PNAS,* 104:5163–68, 2007). Last year, a team at the University of Maryland used this approach—called designer receptors exclusively activated by designer drugs (DREADDs)—to disrupt a mouse's ability to learn to avoid an aggressive male (*J Neurosci,* 35:10773-85, 2015).

This expanding and diverse tool set is crucial for what neuroscientists seek to accomplish. "My hope is that we can actually solve the brain," Boyden says. "And to do that, we have to have a full map, we have to be able to watch it in action, and we have to be able to control it."

—Alison F. Takemura

DAVID PRINCE/STANFORD UNIVERSITY; LESLIE PRICHEP; STEVE JURVETSON/FLICKR

Gene Editing

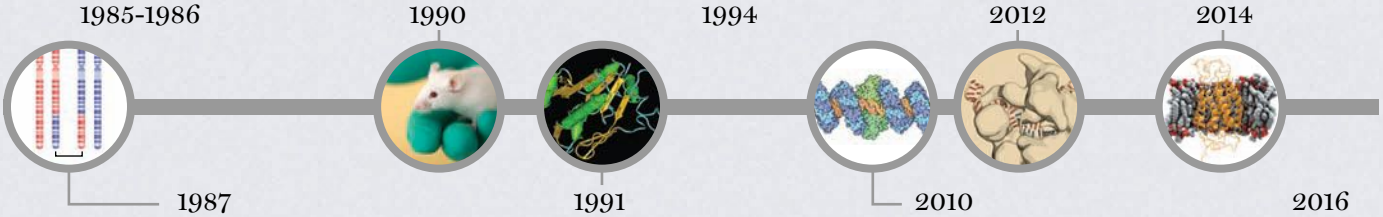
In the mid-1980s, Oliver Smithies, then at the University of Wisconsin–Madison, and Mario Capecchi of the University of Utah independently used homologous recombination—a molecular process to repair broken DNA—to change specific regions of the genome in cultured mouse cells (*Nature,* 317:230-34, 1985; *Cell,* 44:419-28, 1986). The technique involved sandwiching an altered copy of a gene between two regions of code identical to those flanking the endogenous gene, which would be swapped out for its engineered counterpart.

But Capecchi and Smithies couldn't introduce genetic changes into living animals until Martin Evans, now of Cardiff University in the U.K., established a method for culturing mouse embryonic stem cells (ESC). Only ESC or cancer cells could be kept in culture long enough to produce enough genetic material to confirm that homologous recombination had taken place. ESCs also provided a way to create animals harboring genetic modifications, allowing researchers to ask the question, "What does this do in something that runs and smells?" says Dirk Hockemeyer, a stem cell biologist at the University of California, Berkeley.

In 1987, Capecchi reported the targeted disruption of a wild-type gene and Smithies reported the targeted correction of a mutated gene in mouse ESCs. In a series of publications, they brought gene editing into mice, marking the first time anyone had bred a genetically edited animal. Smithies, Capecchi, and Evans shared the 2007 Nobel Prize in Physiology or Medicine for their work.

But the process wasn't easy, says Thomas Doetschman, a molecular biologist at the University of Arizona in Tuscon who was a postdoc in Smithies's lab when the group developed the technology. To obtain a physical copy of a gene of interest required creating a genomic library—a chopped-up mouse genome whose pieces were housed within thousands of bacteriophages, which were cultured inside *E. coli* on oversized petri dishes. "Once you made your library spread out on those plates, those were hot items," Doetschman recalls. Researchers would save the plates in the freezer for years and probe them to find phage-infected bacteria carrying genes of interest. Then they would screen the library, grow up the appropriate bacterial clone, isolate the gene, and assemble a genetic construct—called the targeting vector—which would be transfected into mouse stem cells using electroporation. After confirming the event by PCR or Southern blot, researchers still had to inject ESCs into blastocysts and wait for the mice to cycle through a couple of generations to determine if the cells carrying the editing genome had been adopted.

Much of this grunt work became obsolete in the late 1980s and early 1990s, as the invention and automation of PCR converged with the sequencing and sharing of the entire mouse genome. But the editing process was still inefficient. Homologous recombination requires some sort of DNA synthesis or repair process to occur, making the technique imprecise and hard to direct. As a result, only 1 to 10 cells in a million would pick up the vector and swap out the DNA in the correct spot. In 1994, developmental biologist Maria Jasin and her team at the Sloan Kettering Institute in New York found a way to increase the rate of homologous recombination events 10- to several thousand-fold. The trick was to use an endonuclease that creates dou-



ble-strand breaks in a unique sequence, usually 4–8 base pairs in length, called a restriction site (*PNAS,* 91:6064-68, 1994). Inducing cuts forces the cell to ramp up its repair through homologous recombination. Jasin's team inserted known restriction sites at random spots in the genomes of mouse fibroblasts and used a yeast endonuclease to induce homologous recombination at those sites (*Mol Cell Biol,* 14:8096-106, 1994).

But to make double-strand breaks around a specific gene—to swap out that copy with another version—required that restriction sites flank that gene. Three years earlier, Nikola Pavletich and Carl Pabo, then at Johns Hopkins University, had already published the potential solution, visible in the crystal structure of DNA-binding proteins containing so-called zinc fingers. Pavletich and Pabo found that zinc finger domains each bind a particular sequence of three bases and can be mixed and matched to target desired stretches of DNA (*Science,* 252:809-17, 1991). In 2001, researchers successfully combined an engineered zinc finger protein and a nuclease to make targeted cuts in the DNA of *Xenopus* oocytes (*Mol Cell Biol*, 21:289-97, 2001). Combining this technique with Jasin's work, researchers could not only make double-strand breaks, but make them anywhere in the genome. "Those two things together are what started gene editing," says Charles Gersbach, a molecular biologist at Duke University.

Advancements, accelerated

Zinc finger nucleases (ZFNs) did not catch on like wildfire, however. They were tricky to work with and required an advanced understanding of protein structure and a lot of trial and error, says Raj Chari, a postdoc in geneticist George Church's lab at Harvard University. "Typically, just to get the reagent to even start the experiment, it would take months."

Gersbach's lab was two years into a project to repair a mutated form of the dystrophin gene using ZFNs when, in 2011, one of his graduate students asked to switch to the recently developed transcription activator-like effector nuclease (TALEN) system. Gersbach discouraged him, saying that it was probably not as easy as it looked and that it was wisest to see the zinc finger project through. But the student didn't listen. "Two months later he came back in and said, 'I've got it all working in TALENs now,'" Gersbach recalls.

Like zinc fingers, TALEs are DNA-binding proteins that can be attached to a nuclease (creating a TALEN), but each TALE subunit binds to only a single nucleotide, making them much easier to string together to target specific sequences. "Most of the ZFNs that we made didn't work, and most of the TALENs did," says Gersbach.

When CRISPR/Cas9 became available two years later, Gersbach's students again wanted to switch to the newer technology, and again, he discouraged them. "Luckily, they don't listen to me," he says. In December 2015, his team was one of three that described CRISPR/Cas9-mediated editing of the gene for dystrophin in neonatal and adult mice (*Science,* doi:10.1126/science.aad5143, 2015).

CRISPR/Cas use has recently exploded. In 2012, just 126 publications indexed by Pubmed mentioned the technology; nearly 10 times as many came out in the first six months of this year. The new approach has spurred international discussion about the ethics of human gene editing and high-profile patent disputes between competitors and among collaborators illustrates just how crucial this gene-editing technology is to life science. Above all, it has changed the game of genome tinkering, having already demonstrated potential to edit DNA in cell lines, embryos, and even mice.

"When CRISPR came along it was pretty clear that was going to make life a lot simpler," says Doetschman. Nowadays, when he wants to create a mouse line with an altered gene, in most cases he can find a guide RNA to target the CRISPR proteins to his gene of choice and inject plasmids encoding the RNA and a Cas nuclease directly into a fertilized mouse egg, skipping the in vitro ESC work. "It's [nothing] short of miraculous. It's hard to believe how efficient [CRISPR] is."

—Amanda B. Keener

The Stem Cell Workhorse

Stem cell research was going strong by the late 1990s. Developmental biologists had been using human embryonal carcinoma (EC) cells at the bench since the mid-1980s, but work on embryonic stem cells (ESCs) and embryonic germ cells in the mouse had given researchers hope that they could derive pluripotent cells from humans that wouldn't have the EC cells' abnormal genomes. Leading up to the new millennium, a handful of researchers were working furiously toward this goal.

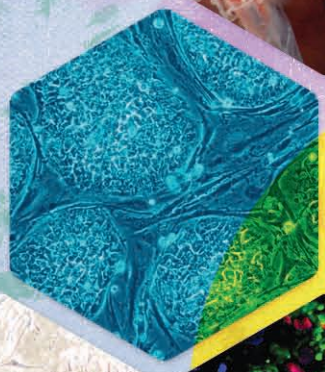
Restrictions to federal funding for human embryo research in the U.S. severely hindered efforts, but in November 1998, two labs supported by private funding from the Geron Corporation succeeded. James Thomson, a developmental biologist at the University of Wisconsin–Madison, and colleagues isolated and cultured stem cells from donated human IVF embryos (*Science,* 282:1145-47, 1998), while another team led by John Gearhart, then at Johns Hopkins University, derived human embryonic germ cells from donated fetal tissue (*PNAS,* 95:13726-31, 1998).

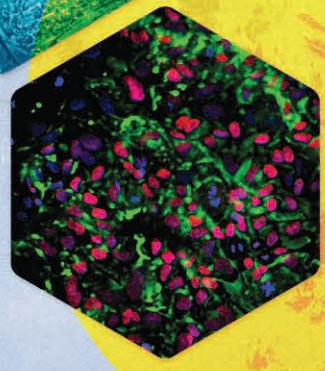
Mining human embryos and fetuses for pluripotent cells incited a public outcry. "It was clear once we published that all hell broke loose," says Gearhart. Two extremist camps rapidly arose. "For some people . . . I was the one who was going to provide all of these cures," he recalls. "And to other people I was the devil—killing babies, all of that." Within months, Gearhart says, he and Thomson were testifying before Congress in defense of their work, while Johns Hopkins removed the signs pointing to Gearhart's lab for his protection and his family endured threats from protestors. To help his trainees understand the debates, Gearhart brought ethicists and philosophers into the lab.

The public backlash continued for years, but the science carried on. Despite the fraught political backdrop, research on pluripotent stem cells has sparked advances in fields from developmental biology to genetics, from drug discovery to regenerative medicine.

Sources of stem cells

When pluripotent stem cells were isolated from mouse embryos in 1981 (*Nature,* 292:154-56; *PNAS,* 78:7634-38), the new cell lines enabled two major advances: the growth of large numbers of cells for studying development, and the creation of genetically engineered mice. "Mouse embryonic stem cells changed the whole of mammalian genetics," says Janet Rossant, a developmental biologist at the Hospital for Sick Children in Toronto. "The ability to take a cell in culture, genetically manipulate it—alter it, knock out genes, add genes, do anything you want—and put it back into the embryo, and have it contribute to the mouse, really was revolutionary."





Over the next 17 years, biologists developed procedures and culture conditions to derive multiple differentiated cell types from mouse ESCs, including hematopoietic cells, muscle cells, and neurons, but researchers struggled to replicate the feat in human cells. With the isolation of pluripotent stem cells from human embryos and fetal tissue by Thomson and Gearhart in 1998, however, the stem cell field entered a realm where human cell therapies seemed attainable.

In the meantime, at the University of Edinburgh's Roslin Institute, embryologist Ian Wilmut and his colleagues had inserted the nucleus of a mammary cell into an ovine egg cell to create Dolly the sheep, demonstrating that the mammalian oocyte contained factors that could reprogram a fully differentiated cell's DNA (*Nature,* 385:810-13, 1997). Later cell-fusion experiments showed that human embryonic stem cells also contained reprogramming factors that could restore the somatic genome to a pluripotent embryonic state (*Science,* 309:1369-73, 2005). Once identified, researchers expected, these reprogramming factors could convert any differentiated cell into an embryonic stem cell–like state—and conveniently sidestep much of the controversy over using human embryos for research.

In Massachusetts, MIT geneticist Rudolf Jaenisch tasked most members of his lab with finding these factors as a side project to their primary research. "It was clear that it would be a very important finding, but it was also clear that it may not work," says Stanford University's Marius Wernig, a postdoc in Jaenisch's lab at the time. But Shinya Yamanaka, a stem cell biologist at Kyoto University, beat Jaenisch's team to the punch. In 2006, Yamanaka and colleagues described four transcription factors—Oct4, Sox2, c-Myc, and Klf4—that could convert mouse fibroblasts to a pluripotent state when their genes were introduced to the cells' genome using a retrovirus (*Cell,* 126:663-76). The results were called induced pluripotent stem cells (iPSCs).

Jaenisch's team already had the genes for the four factors cloned, and began immediate follow-up. "It was a matter of days, literally, to put these things together and repeat the key experiment that Shinya Yamanaka published," recalls Wernig. It worked (*Nature,* 448:318-24, 2007), and the creation of iPSCs from human fibroblasts quickly followed later that year (*Cell,* 131:861-72, 2007; *Science,* 318:1917-20, 2007).

"That's really the secret to the whole field," says Jeanne Loring, director of the Center for Regenerative Medicine at the Scripps Research Institute. "Making iPS cells turned out to be very straightforward."

Streamlining the process

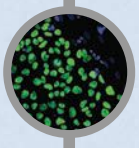
Since the mid-2000s, the number of labs using iPSCs in research has steadily increased. But the process of reverting adult cells to a pluripotent state wasn't perfect, and the danger of tumor formation posed a major obstacle to therapeutic development. Reprogramming cells to a pluripotent state is an inherent risk; one of the tests of pluripotency is whether the cells can create teratomas. Additionally,

1981

Mouse embryonic stem cells isolated

1996

Dolly the sheep is born, demonstrating that the mammalian oocyte has the ability to reprogram the DNA in an adult cell.



1998

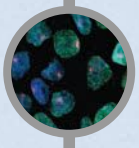
Human embryonic stem cells isolated

2001

U.S. President George W. Bush prohibits federal funding for research using human embryonic stem cell lines derived after August 9, 2001.

2006

Creation of first induced pluripotent stem cells in mice



2007

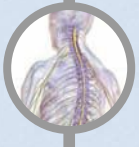
Creation of human iPSCs

2009

The NIH approves an additional 13 human embryonic stem cell lines eligible for federal research funding.

2010

Researchers reprogram mouse fibroblasts directly into neurons.



2010

The Geron Corporation initiates first FDA-approved human embryonic stem cell clinical trial, testing a therapy for spinal cord injury; trial discontinued in 2011

2014

First clinical trial using human iPSCs initiated; trial put on hold in 2015

one of Yamanaka's original factors, c-Myc, is the product of a proto-oncogene that can spur tumor growth in iPSCs (*Nature,* 448:313-17, 2007), and viral vectors raised the possibility that the transcription factors would activate an oncogene when they integrated into the host genome.

Early work on the reprogramming recipes incorporated new factors in place of c-Myc (*Nat Biotech,* 26:101-06, 2008). Researchers have also tried other delivery methods, such as introducing proteins or synthetic mRNA. But these alternatives are still laborious, and most are no more efficient than the retroviral reprogramming—and often less so. A few years after iPSCs were developed, Wernig's team figured out how to skip the pluripotent state altogether, converting directly from one differentiated cell type to another (*Nature,* 463:1035-41, 2010).

Direct conversion may theoretically avoid some of the cancer risks of pluripotent cells, but researchers are also looking to the process as a way to streamline cell generation. "A lot of these differentiation protocols that you use to get a specialized cell type like lung or hepatocyte, they're quite long and cumbersome," says Rossant. "If one could shortcut that and do a direct conversion, it could be very useful."

To the clinic

While some teams worked to improve stem cell conversion at the bench, others have been eyeing ways pluripotent cells could be transformed for the clinic. But cell therapies have been dogged with quality-control concerns, with critics asking how closely the final differentiated cells mirror those in a healthy adult. Working out the details of different developmental pathways has been a challenge, says Gearhart. "Many of us thought we'd be able to do it in a few years, but here we are still struggling 20 years later in some of these lineages."

Nevertheless, stem cell–based regenerative medicine is finally edging closer to clinical application. Labs around the world are using pluripotent cells to generate a variety of therapeutic cell types, such as pancreatic beta cells for diabetes and dopaminergic neurons for Parkinson's. In 2010, the Geron Corporation began the first FDA-approved clinical trial using human ESCs to treat spinal cord injury, though the project was shut down in 2011 due to high costs after enrolling only four patients. The Astellas Institute for Regenerative Medicine (formerly Advanced Cell Technology) is pursuing an ESC treatment for macular degeneration, and launched a Phase 2 clinical trial last year. In 2014, Masayo Takahashi, an ophthalmologist from the RIKEN Center for Developmental Biology, in collaboration with Yamanaka, began the first iPSC clinical trial for a therapy to also treat macular degeneration. The trial was put on hold after the researchers discovered mutations in the cells, but one patient safely received the treatment, and the study team plans to resume the trial.

Dozens of other pluripotent stem cell–based therapies have shown promise in preclinical studies, but so far, a definitive clinical success remains elusive. To break open the field of regenerative medicine for good, "we need to have a trial succeed," Loring says.

—Karen Zusi

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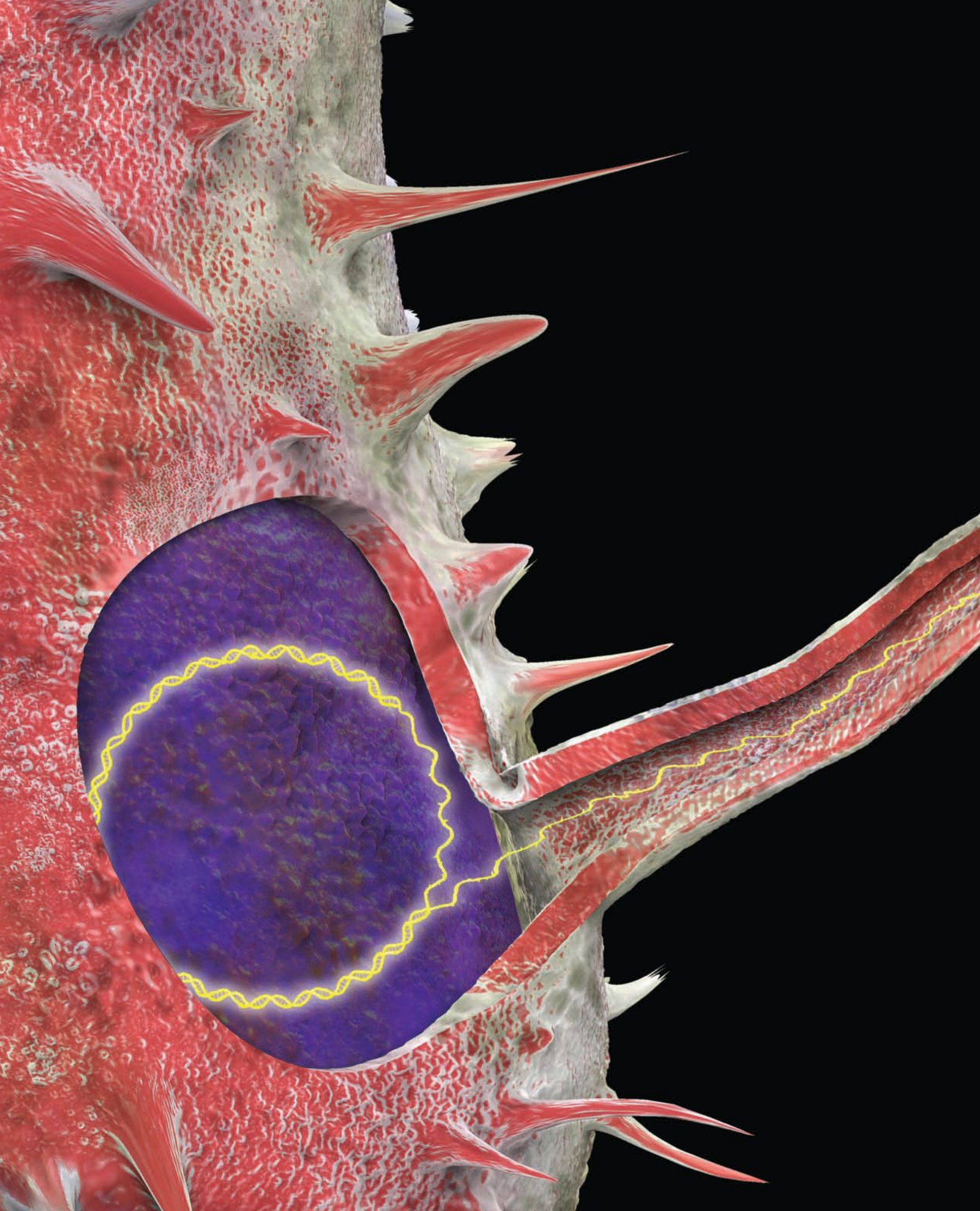


Bacterial Cut-and-Paste

Bacteria inhabit most tissues in the human body, and genes from some of these microbes have made their way to the human genome. Could this genetic transfer contribute to diseases such as cancer?

BY KELLY ROBINSON AND JULIE DUNNING HOTOPP

Before we understood that DNA was the genetic code, scientists knew that bacteria transferred it between cells. In 1928, 25 years before the structure of DNA was solved, British bacteriologist Frederick Griffith demonstrated that live, nonvirulent bacteria could transform into virulent microbes after being incubated with a heat-killed virulent strain. Fifteen years later, a trio of researchers at the Rockefeller Institute for Medical Research (now Rockefeller University), Oswald Avery, Colin MacLeod, and Maclyn McCarty, demonstrated that this transformation was mediated by DNA. Even dead bacteria, it seemed, could share their genes.

This DNA-sharing process, known as horizontal or lateral gene transfer

(LGT), is now understood to occur by the direct movement of DNA between two organisms. Almost all bacterial genomes show evidence of past LGT events, and the phenomenon is known to have profound effects on microbial biology, from spreading antibiotic resistance genes to creating new pathways for degrading chemicals. But LGT is not limited to bacteria. Scientists now recognize that microbes transfer DNA to the plants, fungi, and animals they infect or reside in, and conversely, human long interspersed elements (LINEs) have been found in bacterial genomes. Moreover, researchers have documented LGT from fungi to insects and from algae to sea slugs. There is reason to believe that any two major groups of organisms—including humans—can share their genetic codes.

People have long been intrigued by the prospect of foreign DNA within our own genomes. Human genomes harbor evidence of beneficial LGTs from bacteria in the recent past, and there is evidence that transfers may occur regularly between resident bacteria and somatic cells of the body. How commonly bacteria-animal LGT occurs is unclear, as are the mechanisms of these transfers. But if LGTs induce harmful mutations, they may be an unrecognized cause of disease.

Gene swap

Bacteria are a genomically promiscuous bunch. They do not reproduce sexually but are among the most genetically varied species because they are constantly exchanging bits of their genetic code via LGT. Their diversity has allowed them to adapt to every ecological niche on the planet, from deep-sea hydrothermal vents to the frozen lakes of Antarctica, from rock crevices to our own intestines. LGT between bacteria has been categorized as transformation by free DNA (genetic material is released into the environment by bacteria and taken up by living microbes, as in Griffith's experiment), transduction by viruses, and direct cell-cell transfer through conjugation.

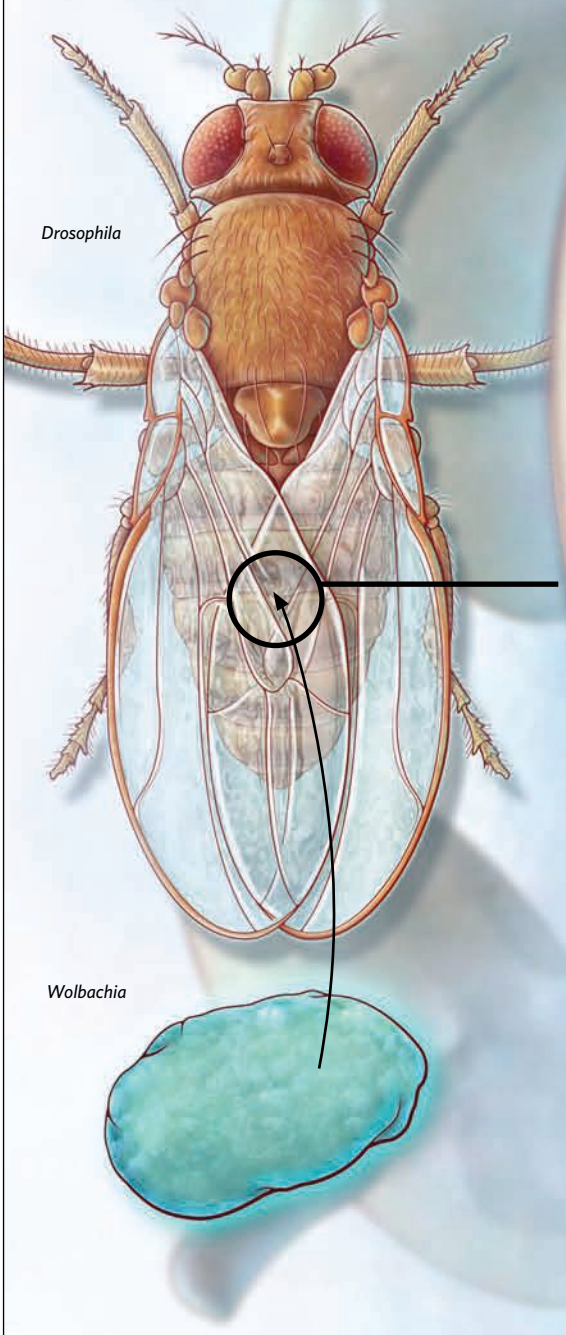
The mechanisms of transfer from bacteria to other organisms are less clear, but are likely similar. Bacteria's type IV secretion system is a syringe-like protein known to inject molecules from bacteria into their host cell though cell-cell contact. It is an important mediator of LGT between *Agrobacterium* and plants in the wild, as well as in the lab, where it can be used to create genetically modified crops and can even mediate transfer between *Agrobacterium* and human cells. Using whole genome sequencing, researchers have found that the genomes of numerous insects and nematode worms sometimes contain DNA from microbes inhabiting or infecting their bodies. Some species contain vast arrays of *Wolbachia* endosymbiont DNA, for example—up to many complete copies of the bacterial genome. (See illustration at right.)

Almost all bacterial genomes show evidence of past LGT events, and the phenomenon is known to have profound effects on microbial biology.

These large LGTs can be nearly identical in sequence to the endosymbiont genome, suggesting that they happened quite recently. Some insect species carry remnants of much older gene transfers that were beneficial to the recipient species and have been selected for over time. The coffee berry borer, for example, coopted a bacterial mannanase gene that allows it to eat coffee berries.[1] Coopted bacterial mannanase genes may also underlie crop destruction caused by the invasive brown marmorated stink bug.[2] And aphids synthesize their own carotenoids using genes transferred from fungi to produce a colorful appearance important to defense.[3] As more examples of LGT among diverse organisms crop up in the literature, it's only natural to

GENE SWAP

Horizontal or lateral gene transfer (LGT) is a regular event among bacteria, and research over the past decade has shown that microbes can also transfer their DNA to multicellular hosts. One of the most well studied examples of LGT between microbe and animal is the transfer of DNA from an intracellular *Wolbachia* endosymbiont to its *Drosophila* host.

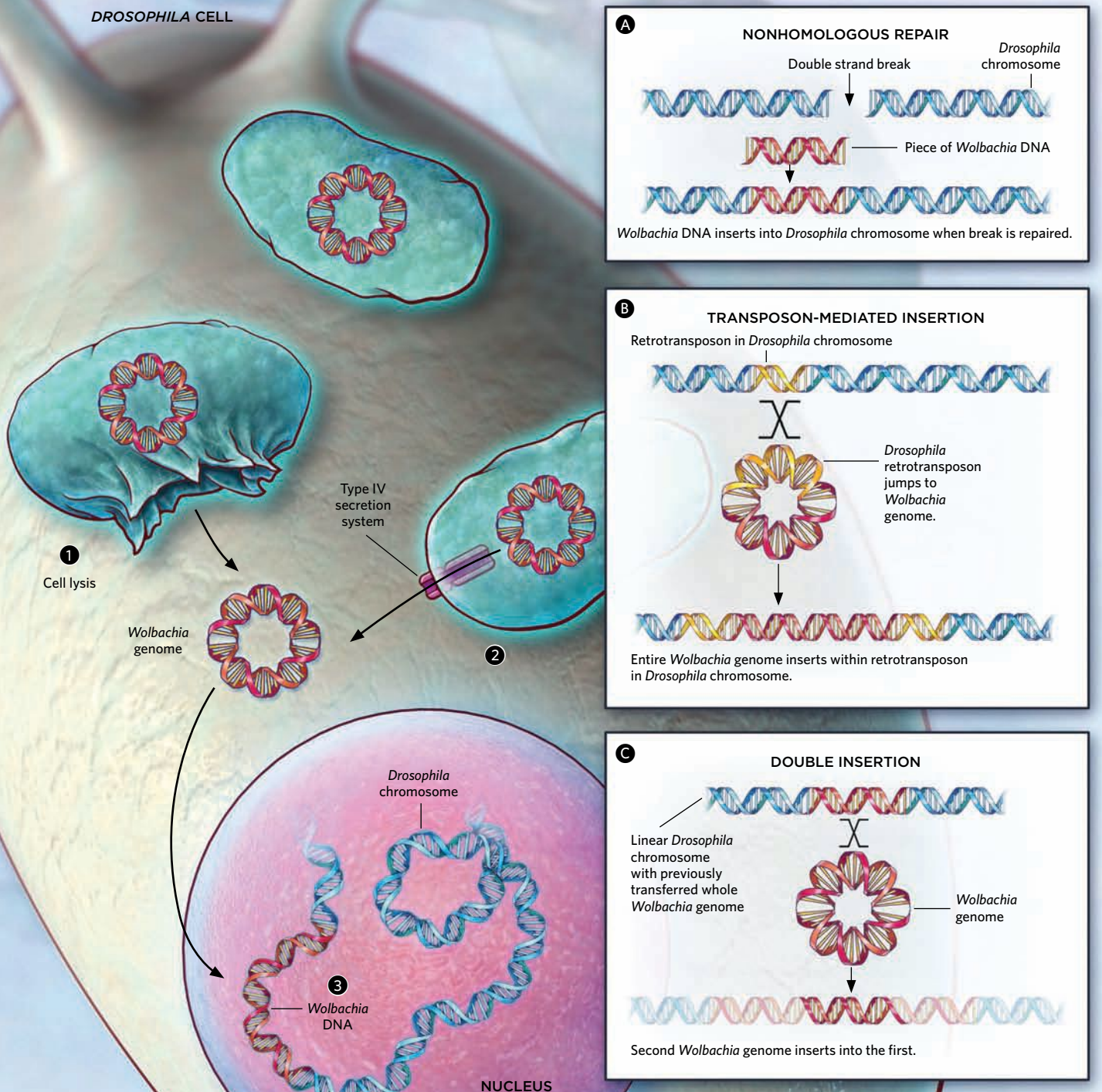


WOLBACHIA GENE TRANSFER

Bacterial DNA may enter the cytoplasm of the *Drosophila* cell via bacterial cell lysis ❶ or a type IV secretion system ❷. Once in the cytoplasm, some or all of the bacterial genome can be integrated into the fly genome ❸. As long as the host remains colonized by its *Wolbachia* endosymbiont, LGT can continue and *Wolbachia* DNA can accumulate in the *Drosophila* genome.

MECHANISMS OF DNA INTEGRATION

Just how *Wolbachia* DNA inserts itself into the *Drosophila* genome is unclear. Three likely mechanisms include non-homologous repair of a double-strand break Ⓐ, insertion of a fly retrotransposon into the *Wolbachia* genome followed by homologous recombination with another copy of the retrotransposon in the fly genome Ⓑ, and/or homologous recombination with a prior nuclear *Wolbachia* transfer (nuwt) Ⓒ.



focus on the human angle. Does it occur in us, and if so, how often, and what are the consequences?

LGT in humans

The extent and importance of LGT in vertebrate animals is less clear, in part because fewer of their genomes have been sequenced, and/or analyzed with suitable methods, compared with those of invertebrates. One vertebrate species whose genome has been extensively studied—humans—has yielded solid evidence of ancient LGT events.

In 2001, the first draft sequence of the human genome was suggested to have 223 LGT-derived regions that were not present in other species' genomes that had been sequenced at that time.[4] Some researchers quickly disputed this number as an overestimate, even suggesting that all of the proposed LGTs were more likely explained through alternative mechanisms such as gene loss or convergent evolution.[5] A new analysis published last year by Alastair Crisp of the University of Cambridge and colleagues found more than 130 traces of possible LGT events in the human genome—including the presence of fungal hyaluronan synthases, a fat mass and obesity associated gene (*FTO*), and the gene responsible for blood types (*ABO*). But most, if not all, of the identified events predate the human and primate lineages and were identified because the researchers chose to no longer limit the results to LGTs that exist only in humans and not in other animal species.[6]

In order for a nonhuman gene to appear in the genomes of many people, however, the LGT needs to occur in the germline so that it can be passed to future generations; and it has to confer some benefit to the host. Such LGTs may be rare, because humans may not experience strong selection for new functions in our genome, and because our germ cells are thought to be protected from other organisms and their DNA. However, LGT might be possible in the somatic human genome; such insertional mutations would be very difficult to detect, though, without sequencing large numbers of human cells.

Once they are present in the human somatic genome, it's not hard to imagine how LGT insertions could cause disease. In fact, while definitive evidence of recent LGT in humans is still lacking, there are other types of DNA transfer that are well known to negatively impact humans. For example, human papillomavirus (HPV) is the cause of 80 percent to 100 percent of cervical cancers. The virus can integrate into the chromosomes of cervical cells, and if the integration is incomplete, certain HPV proteins can become unregulated, leading to disruption of apoptosis, an increase in cell proliferation, and ultimately cancer. Likewise, hepatitis B virus (HBV) causes hepatocellular cancer and has been found to insert its DNA into infected hepatocytes as the cells regenerate. HBV recurrently integrates its viral enhancer gene and its core gene into cancer-related genes, causing increased cell growth and survival, two hallmarks of cancer.[7]

Studies suggest that LGT events can and do occur in human tissues, perhaps with devastating consequences.

Given the known risk of such integrations, we have focused on identifying LGT of bacterial DNA in the human genome. We knew that we needed to look at data from a large number of individuals, so we relied on publicly available human sequence data from the original public and private human genome projects and the 1000 Genomes Project. We quickly realized that if an LGT happened in a terminally differentiated cell that no longer replicates its DNA, it would exist in only one copy, and we would never be able to distinguish it from noise during sequencing. So we turned to tumors. We figured that, should an insertion occur in a progenitor cell of the tumor, it should be propagated in the tumor and be detected multiple times.

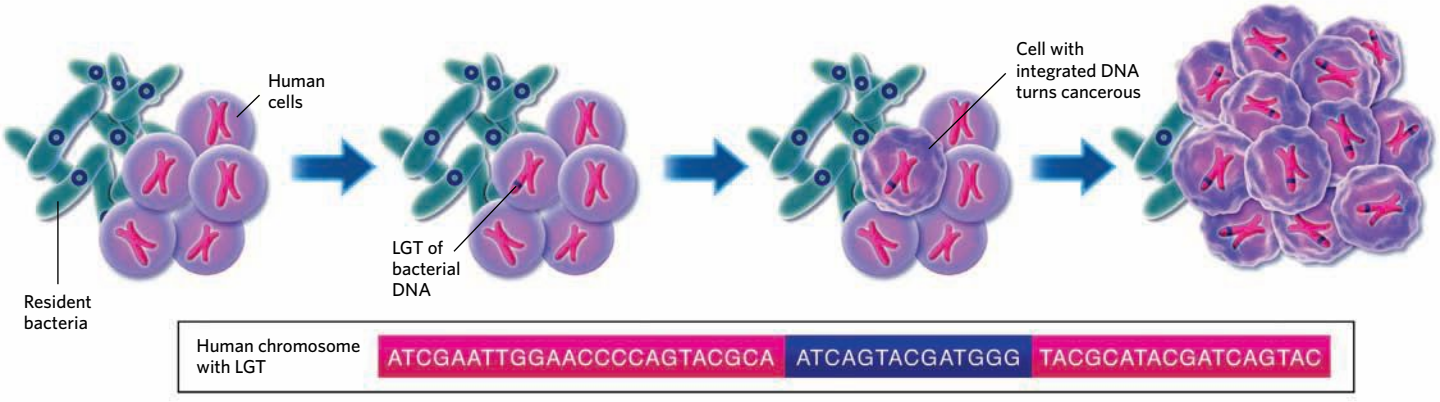
We analyzed genome sequence data from nine different tumor types from Cancer Genome Atlas projects and used bioinformatics tools to identify potential DNA integrations. In results published in 2013, we found sequences from *Acinetobacter* species in acute myeloid leukemia (AML) samples and from *Pseudomonas* species in stomach adenocarcinoma (STAD) samples. There were recurrent insertions in cancer-related genes in the STAD samples.[8]

In both the AML and STAD cancer samples, we only identified evidence of bacterial 16S and 23S rRNA fragments integrating into the human genome. Karsten Sieber, then a graduate student in the Dunning Hotopp lab, created models of the STAD integrations in cancer-related genes and observed that these pieces of the rRNA genes contain secondary structures that form numerous stem-loops, or hairpin loops. These integrations occur in the 5'-untranslated region (5'-UTR) of the cancer-related genes, meaning that they are transcribed but not translated. The stem-loops predicted in the inserted rRNA gene fragments could alter the secondary structures of the transcripts, thereby disrupting transcription and/or translation. We also noticed that the putative STAD integrations occur in G-rich regions of the cancer-related genes, which can also be important for gene regulation.[9]

Ying Xu at the University of Georgia has also identified LGT events in human tumors. His team looked for evidence of genetic material from *Helicobacter pylori* bacteria and the Epstein-Barr virus, both of which have been associated with gastric cancer. The researchers identified *H. pylori* integrations in 36 genes in the gastric samples, with more integrations present in the tumors relative to controls.[10] Chronic infection with *H. pylori* can cause double-strand DNA breaks, and human cells may "heal" these double-strand breaks by inserting pieces of stray DNA. Often this is nuclear or mitochondrial DNA, but if bacterial DNA is present, including *H. pylori* DNA, it could become integrated. These integrations could

LGT-CAUSED CARCINOGENESIS?

If DNA is transferred from resident bacteria to human somatic cells, the integration risks transforming normal cells into cancerous ones.



therefore be a side effect, rather than a cause, of cancer.

It's unclear how bacterial DNA evades the human immune system, which recognizes most forms of nucleic acids. But these studies suggest that LGT events can and do occur in human tissues, perhaps with devastating consequences. So far, these are the only reported cases of bacterial DNA integrations in human cancers. Whether such events cause cancer, and if so, how commonly, remains to be seen.

A steep road ahead

A number of challenges face researchers hoping to assess the presence and impact of bacterial DNA integrations in the genomes of human cells. A thorough study of this sort is still expensive, and after the samples have been sequenced it takes significant resources to develop, implement, and run a computational tool to identify LGT.

Contamination also remains a barrier. This was an issue in an analysis of the human genome in 2001, and it continues to be a problem today. DNA extraction kits have been shown to contain bacterial nucleic acids. Contaminants can also be introduced via sample handling, from reagents, and during sequencing. During the process of creating the DNA library to be sequenced, chimeras that look like bacterial DNA integrations might form. Sure enough, earlier this year, researchers found that the extent of LGT in the tardigrade genome was initially overestimated; some proposed LGTs likely arose from the genomes of bacterial contaminants and not from the tardigrade genome itself.[11]

Despite skepticism from some corners of the scientific community and the difficulties of studying bacterial DNA integrations, we believe that LGTs are an important form of insertional mutagenesis. Perhaps now that putative bacterial DNA integrations have been identified in cancer, more researchers will look for these mutations in other diseases. A bacterial DNA integration that occurs in a human cell and leads to the expression of a bacterial compound recognized by the human immune system has the potential to trigger autoimmune disease, for example. Further research on the occurrence and consequences of LGT in human cells will likely reveal the phenomenon to be much more common and important than currently appreciated. ■

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The Literature

GENETICS & GENOMICS

Running Through Stop Signs

THE PAPER

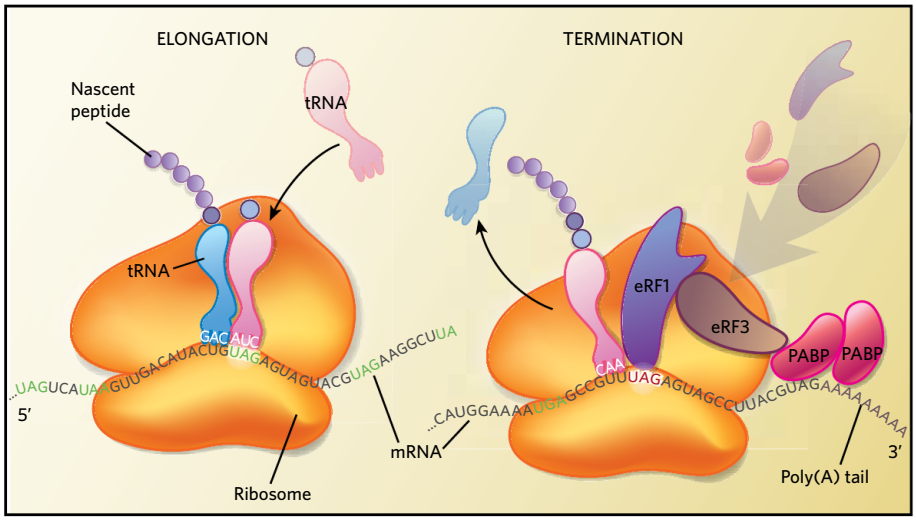
E.C. Swart et al., "Genetic codes with no dedicated stop codon: Context-dependent translation termination," *Cell*, 166:691-702, 2016.

The genetic code—the digital set of instructions often laid out in tidy textbook tables that tells the ribosome how to build a peptide—is identical in most eukaryotes. But as with most rules, there are exceptions. During a recent project on genome rearrangement in ciliates, Mariusz Nowacki, a cell biologist at the University of Bern in Switzerland, and his team stumbled across two striking deviants.

Ciliates, complex protozoans with two nuclei, are known to translate RNA transcripts in unorthodox ways. Nowacki's team, however, discovered that *Condylostoma magnum* and an unclassified *Parduczia* species had gone even further, reassigning all of the traditional "stop" codons (UGA, UAA, and UAG) to amino acids. "It didn't make sense in the beginning," says Nowacki. "Nobody would expect that there would be a stopless genetic code."

Puzzled, Nowacki's team wanted to find out what the ciliates' actual stop codons looked like. The group turned to transcripts of the ciliates' histone proteins because their sequences are highly conserved across all eukaryotes. Using protein mass spectrometry and ribosome profiling, the researchers determined that the *Parduczia* species always interpreted UAA and UAG as glutamine codons, but read UGA as a tryptophan codon in some cases and as a stop codon in other cases. Even stranger, in *C. magnum* all three traditional stop codons functioned as either a stop or an amino acid signal.

"For the cells to survive, they have to be able to resolve this ambiguity," says Nowacki. The data showed that the ribosomes somehow knew to interpret the same combination of three nucleotides as either "stop" or "go" in the appropriate context—so Nowacki's team started looking for contextual clues.



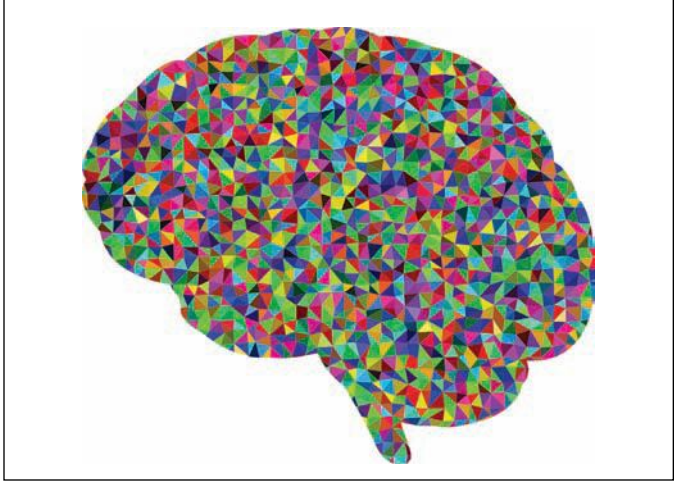
STOP AND GO SIGNALS: Certain ciliates use traditional stop codons ambiguously during translation. Sometimes these three-base-long RNA sequences code for an amino acid (green, left), and sometimes induce translation termination (red, right). Researchers propose that at the end of the mRNA coding sequence, the ribosome bumps up against proteins involved in termination—namely, eukaryotic release factors (eRF1 and eRF3) and poly(A) binding proteins (PABP)—thereby indicating that a stop codon means stop.

The researchers discovered that the appearance of the ambiguous codons declined dramatically near the end of transcripts, cutting down on the potential confusion. Additionally, *C. magnum* and the *Parduczia* species had a remarkably short length of untranslated mRNA between the translated part of the transcript and the 3' poly(A) tail, compared with other eukaryotes. The team suggests that proteins coating or interacting with the poly(A) tail may act as roadblocks to translation when the ribosome bumps up against them. In yeast, poly(A)-binding proteins have been shown to play a role in translation termination.

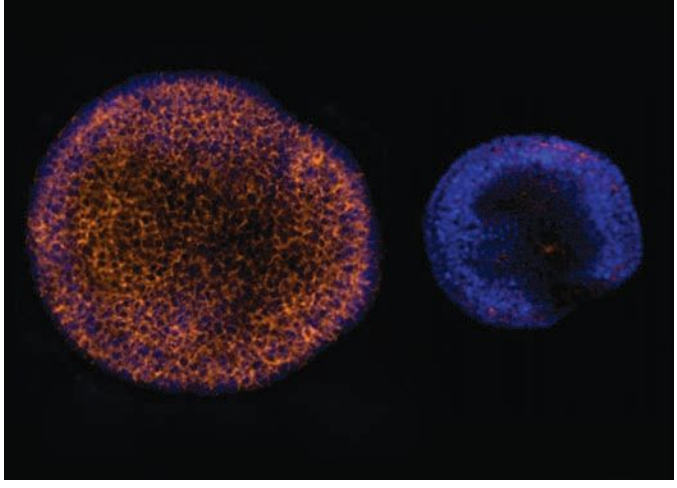
"I think it's a very good explanation," says Andre Cavalcanti, a molecular biologist at Pomona College in California, though it raises the question of whether the codon reassignment or the short untranslated region (UTR) evolved first. "Not every ciliate that reassigns the genetic code has UTRs as short as these, which seems to be a requirement of the model," he added in an email to *The Scientist*. "Different ciliates might adapt in different ways."

To test its hypothesis, Nowacki's team is planning to tinker with the length of the UTR in *C. magnum*. If the researchers can push the poly(A) tail farther away from the true stop codon, Nowacki predicts, they may see more readthrough.

"We were able to identify a novel biology in sea creatures, in creatures that were never looked at before," says Nowacki. "It shows that [the genetic code] is not necessarily frozen and unambiguous." —Karen Zusi



EXCITED LIGHT: Neurons stimulated with the neurotransmitter glutamate produce biophotons, ultraweak pulses of light.



SIMMER DOWN: A mouse adenoma organoid treated with an NSAID (right) has less parainflammation (orange marker; control on the left).

NEUROSCIENCE

Spectrum of Intelligence

THE PAPER

Z. Wang et al., "Human high intelligence is involved in spectral redshift of biophotonic activities in the brain," *PNAS*, 113:8753–58, 2016.

BRAIN AGLOW

Neurons can emit ultraweak photons when stimulated by the abundant neurotransmitter glutamate, and experiments suggest that the emitted light can travel along rat nerve fibers. Yet most researchers don't believe there's enough evidence to say whether these so-called biophotons act as communication signals, according to Michal Cifra, who studies the phenomenon at the Institute of Photonics and Electronics in the Czech Academy of Sciences.

CATCHING SOME RAYS

To test for a biological role for biophotons, a team in China analyzed brain slices from six species of roughly increasing intelligence: bullfrog, chicken, mouse, pig, rhesus monkey, and human. The group doused the tissues with the neurotransmitter glutamate and determined the photon emissions' wavelengths using a newly designed spectrometer.

SPECTRAL SHIFT

For the most part, the more intelligent the species, the longer the biophoton wavelength: from about 600 nm among bullfrogs to 700 nm among humans, on average. (Chickens' biophotons had longer wavelengths than mice's, suggesting to the authors that chickens are smarter.) Because red-shifted light has lower energy, this spectral slide could help species, if they do use light biologically, to expend less energy, says study coauthor Jiapei Dai of South-Central University for Nationalities in Wuhan. "It's a correlation," says Dai. "But maybe [it] gives an explanation for human high intelligence."

HARSHER LIGHT

Cifra and Vahid Salari, who studies biophotons at Isfahan University of Technology in Tehran, concur that the findings are novel. But the team's conclusions overreach. Cifra doubts "whether this spectral shift is [really] caused by some evolutionary function, or [if] it is just a coincidental artifact of having tissues of differing molecular content." —Alison F. Takemura

CELL & MOLECULAR BIOLOGY

Slow Burn

THE PAPER

D. Aran et al., "Widespread parainflammation in human cancer," *Genome Biol*, 17:145, 2016.

SMOLDERING THREAT

Researchers in Israel were trying to understand why a particular mouse model was so unusually cancer-prone once it suffered a mutation in the tumor suppressor gene *p53*. They noticed that the animal's epithelial tissue showed signs of low-grade inflammation, undetectable by normal hallmarks such as white blood cell recruitment. Intriguingly, treating the mutated mouse with a nonsteroidal anti-inflammatory drug (NSAID) reduced its risk of cancer—an effect observed in some human studies as well.

SMOKE SIGNALS

To explore what was driving this so-called parainflammation (PI), the team recently collaborated with Dvir Aran of the University of California, San Francisco, and colleagues to identify its genetic signature, and found an upregulation of 40 genes. The researchers then analyzed gene expression data from more than 800 human cancer cell lines and from about 6,500 patient tumor samples, including 18 cancer types, to uncover whether any harbored this signature.

STRIKING A MATCH

By scoring gene expression data based on their similarity to the PI signature, the team found four cancers—including lung and pancreatic adenocarcinomas—with notably higher tallies. Mutations in *p53* were also associated with a higher PI score. The prognoses for these cancers were dramatically worse, suggesting PI might exacerbate cancer progression.

COOLING CANCER

When the team treated mouse adenoma organoids and two human cancer cell lines with an NSAID, the expression of PI genes fell by more than half. The findings could translate to the clinic, by helping to identify patients who would benefit from NSAID treatment, says Michael Karin, who studies inflammation in colorectal cancer at UC San Diego. "Right now, nobody is stratifying the patients," he says. —Alison F. Takemura

PIXABAY, GDJ; HEBREW UNIVERSITY OF JERUSALEM, AUDREY LASRY AND *GENOME BIOL*, 17:145, 2016

Curious George

George Church has consistently positioned himself at genomics' leading edge.

BY ANNA AZVOLINSKY

In 1973, after trying out two other groups, George Church finally found the perfect fit in Sung-Hou Kim's X-ray crystallography lab, where he spent his second (and final) year as a Duke University undergraduate. "It was all of the things I was interested in—physics, math, biology, chemistry, and computers—in one. Kim had just come from MIT and was young and full of energy," says Church, now a professor of genetics at Harvard University. "He saw a spark in me that few people had noticed before, and he treated me almost as an equal. I started to blossom in that lab."

Church knew that he wanted to go to graduate school, and had already applied to the microbiology PhD program at Duke when he began to work with Kim. "I thought I should stay at the same university because I was young and immature." Church began his PhD at Duke while continuing to work alongside Kim, with whom he would soon publish five papers, including one on modeling DNA-protein interactions and another on the three-dimensional structure of transfer RNAs. Twelve months into his microbiology PhD, Church decided instead to pursue a degree in biochemistry, but was booted out at the beginning of 1976. The biochemistry curriculum didn't capture his interest any more than microbiology's had, and after he'd flunked two courses, even Kim could not convince the university that Church should remain a student there. "I loved the research, but didn't like the courses because I had already taken them as an undergraduate.

> "In the 1990s, a big fraction of my lab was dedicated to computational biology, but we slowly started regressing back to my love of advancing technologies."

"I then became a technician in Kim's lab and planned to do that for the rest of my life until one day Sung-Hou said to me, 'I don't think you are cut out to be a technician, you have too many ideas. You should apply to graduate school.'" In 1977, Church says he did so halfheartedly, applying only to Harvard University's molecular biology program, "which was crazy for someone who had flunked out of Duke." To his surprise, he got in.

Here, Church describes his first experiment, feeding prescription drugs to tadpoles; his reputation in graduate school; and an early epiphany that drove him to develop basic technologies to benefit all biologists.

CHURCH CHALLENGED

Frogs as guinea pigs. Church was born in Florida in 1954 and adopted by his mother's third husband at age 9, taking his surname. "He was a physician, and I was always fascinated by the things in his medical bag. He loved giving people thyroid hormone, so I got it into my head somehow that frogs were responsive to thyroid hormone. I probably didn't read this in a book of experiments because I had mild dyslexia," says Church. The 13-year-old Church added ground-up hormone pills to the water of a group of tadpoles and compared their growth to that of a group that developed in untreated water. "The hormones accelerated their growth considerably. I remember being excited about the result and presented this in my science class. This was a real experiment—with a control, even—in contrast with the usual emphasis then in science class on facts and not experiments."

Nonstop science. In 1968, Church was sent to the Phillips Academy in Andover, Massachusetts, where he dove deeply into science. "It was an incredible place to learn," he says. He kept a collection of carnivorous plants that he tried to make grow into giants by spiking their water with gibberellic acid, a hormone found in plant and fungi species. After completing the chemistry curriculum, Church was given independent access to the chemistry lab. "My favorite thing was to ask the chemistry professor to pull a random chemical off the shelf to see how fast I could identify it—it was like being a detective. Eventually he started giving me mixtures of chemicals." Church, also drawn to computers and robots, discovered a lone computer in the basement of the math building during his freshman year. Using programming books and eavesdropping on conversations of fourth-year students taking computer labs, he taught himself how to program.

Fast track. Church entered Duke in 1972. "I wanted to go to the best warm-weather school I could get into," he says. He placed out of one year's worth of math and science courses and enrolled in summer courses and graduate-level courses his freshman year, sneaking the permission slip for a virology course in between other forms the professor had to sign. Church earned two undergraduate degrees in two years. "I was in a rush because I felt that all of the college courses were baby courses and because I had to pay for college. I saw this as my route to economic independence."

Confessions of a wallflower. The summer before starting graduate school at Harvard University, Church lived in Boston, read molecular biology papers, and planned experiments, including techniques to improve DNA sequencing. He had already decided he wanted to join the lab of Walter Gilbert, one of the developers of DNA sequencing techniques, for which he would receive the Nobel Prize three years into Church's time in his lab. In one course that

PHOTO BY SETH KROLL

GEORGE CHURCH

Professor of Genetics, Harvard Medical School, Boston, MA
Senior Associate, Broad Institute of MIT and Harvard

Greatest Hits

- Developed the first direct genome sequencing and DNA multiplexing methods that led to the first bacterial genome sequence in 1994 and, in 2003, to next-generation methods
- Spearheaded the Personal Genome Project, a way to engage the public in genomic and health data sharing
- As part of the BRAIN initiative, developed ways to encode data in DNA formats, including temporal records of events in living cells
- Intertwined genome reading and writing technologies that led to the largest (~4 million base pairs) synthetically engineered (recoded *E. coli*) genome to date
- Pioneered applications of CRISPR for organ transplants, aging reversal, and gene drives to eliminate malaria and Lyme disease

fall, the professor spent several slides explaining a paper on which Church was the first author. "He had no clue that the author of that paper was in his class. That made me a bit more secure, but I was still full of insecurity. I was very, very shy that year. There was a *Mad* magazine type of student publication, and one of the issues had a matching game. In one column it said '3 words a day' and that matched to 'George' in the second column. That was my reputation."

CHURCH CHARGES

Baby steps. Church's first foray into technology development was to write sequencing software during a rotation in Don Wiley's Harvard lab after helping to sequence the pBR322 plasmid with Greg Sutcliffe. "In a way, [this was] the first real synthetic biology," Church says of the artificially constructed cloning vector. "I kept trying to develop technology rather than following protocols." In Gilbert's lab, Church worked on yeast mitochondrial introns, but "it was such an obscure field that even if I did it perfectly, I knew very few would really care. I thought that if I was going to put that much work into something, I wanted that to be basic enabling technology that all biologists could benefit from." So Church set out to develop new sequencing methods. "I would come home to my girlfriend (now my wife) and say, 'This is a really great day, I got a factor of 2 improvement in signal to noise. I have 10,000-fold more to go.' I never felt frustrated or nervous. The factor of 2 would eventually turn into 10,000."

Four years later, in 1984, Church published his direct genomic sequencing method, which extended Southern blotting to a new level of sensitivity and didn't require cloning or amplification. Church also came up with the concept of increasing DNA sequencing throughput: mixing many DNA pieces in the same tube and reprobing and reimaging, concepts of barcoding and multiplexing that he published in 1988. These early tools later contributed to the automation and later generations of genomic sequencing.

Technology-focused. Church received a PhD in 1984, and, after a brief time at Biogen, joined Gail Martin's lab at the University of California, San Francisco, to work on embryonic stem cells. He produced no publications, but "became comfortable with embryology and started the technology to read genomes and transcriptomes," which he felt were missing, yet crucial, for stem cell studies. Church joined Harvard's faculty in 1986, aiming to improve technologies for reading, writing, and testing genomes.

A year later, he received one of the first Human Genome Project grants. But while others on the project wanted to plow through the sequencing using existing, expensive technologies, Church thought the goal should be to streamline sequencing and lower its costs.

"In the 1990s, a big fraction of my lab was dedicated to computational biology, but we slowly started regressing back to my love of advancing technologies," says Church. Among the lab's first contributions to sequencing was a method for clonally amplifying DNA in situ, developed in 1999 by an MIT engineering student. "This was something that no biologist in my lab wanted to touch," says Church. In 2005, his lab optimized the technique into next-generation sequencing (NGS) and applied the approach to sequence a lab-evolved *E. coli* genome. By 2009, NGS brought down the cost of reading genomes a millionfold, according to Church.

Resurrecting species. In 2008, researchers used NGS to sequence a large portion of the mammoth genome from a recovered hair sample, discovering that the extinct creature is closely related to the Asian elephant. After commenting on the effort in *The New York Times*, Church says he began to think seriously about attempting to resurrect the species. "Like all kids, I was fascinated by large extinct creatures, and I tended to like the furry ones better," he says. The project is small compared to others in his lab, but Church has plenty of volunteers wanting to tweak the elephant genome to resemble that of the mammoth. With private funding, the team has already replaced 15 genes in an elephant cell line with mammoth ones, including those that code for a cold-climate hemoglobin, long hair, small ears, and subdermal fat storage. "We have been developing the methods to engineer genomes of embryos in pigs. But for elephants, we lack key induced pluripotent elephant cells and reproductive technology," says Church. The lab is also trying to develop organoid models to grow reproductive organs to eventually study mammalian—including elephant—development.

Due credit. The story of who turned CRISPR/Cas9 into a precise editing technology is complex, Church contends. After Emmanuelle Charpentier's and Jennifer Doudna's laboratories together demonstrated the system could be simplified and programmed to cut DNA in cell-free systems, Prashant Mali and Luhan Yang in Church's lab demonstrated that Cas9 could be used instead for homologous recombination and in human induced pluripotent stem cells. The publication came out in the same issue of *Science* as a paper that reported similar findings by Church lab alumni, Le Cong and Feng Zhang, at MIT. For Church, CRISPR is just one of the latest genome engineering advances that include next-gen sequencing, synthesis, and large DNA assembly methods.

Creating new biology. In August, Church's lab published a paper in *Science* that he describes as "the largest and most radical genome engineering project [to date]." The lab designed a 3.97-megabase-pair *E. coli* genome, replacing seven amino-acid codons with non-canonical synonymous alternative ones. When completely synthesized, the genome should have 62,214 codon replacements. The work is an improvement on a 2013 study in which Church and his colleagues swapped one codon for a synonymous one throughout the entire *E coli* genome. In the new work, the seven targeted codons were replaced in 63 percent of the synthetic genome. The researchers designed an *E. coli* genome with 87 50-kilobase-long segments containing the codon replacements and had commercial companies synthesize 3-kilobase-long fragments, which his lab then assembled into 50-kilobase fragments in yeast. So far, the team has replaced 55 of the wild-type segments with the novel ones and tested bacterial viability. Church's lab is now working to test the rest of the genomic segments. The ultimate goal, according to Church, is to create a biocontained multivirus-resistant strain that is better suited for industry applications such as protein and chemical production. "This is also a good model. If you can get this to work in *E. coli*, you can get it to work in a lot of other organisms," says Church.

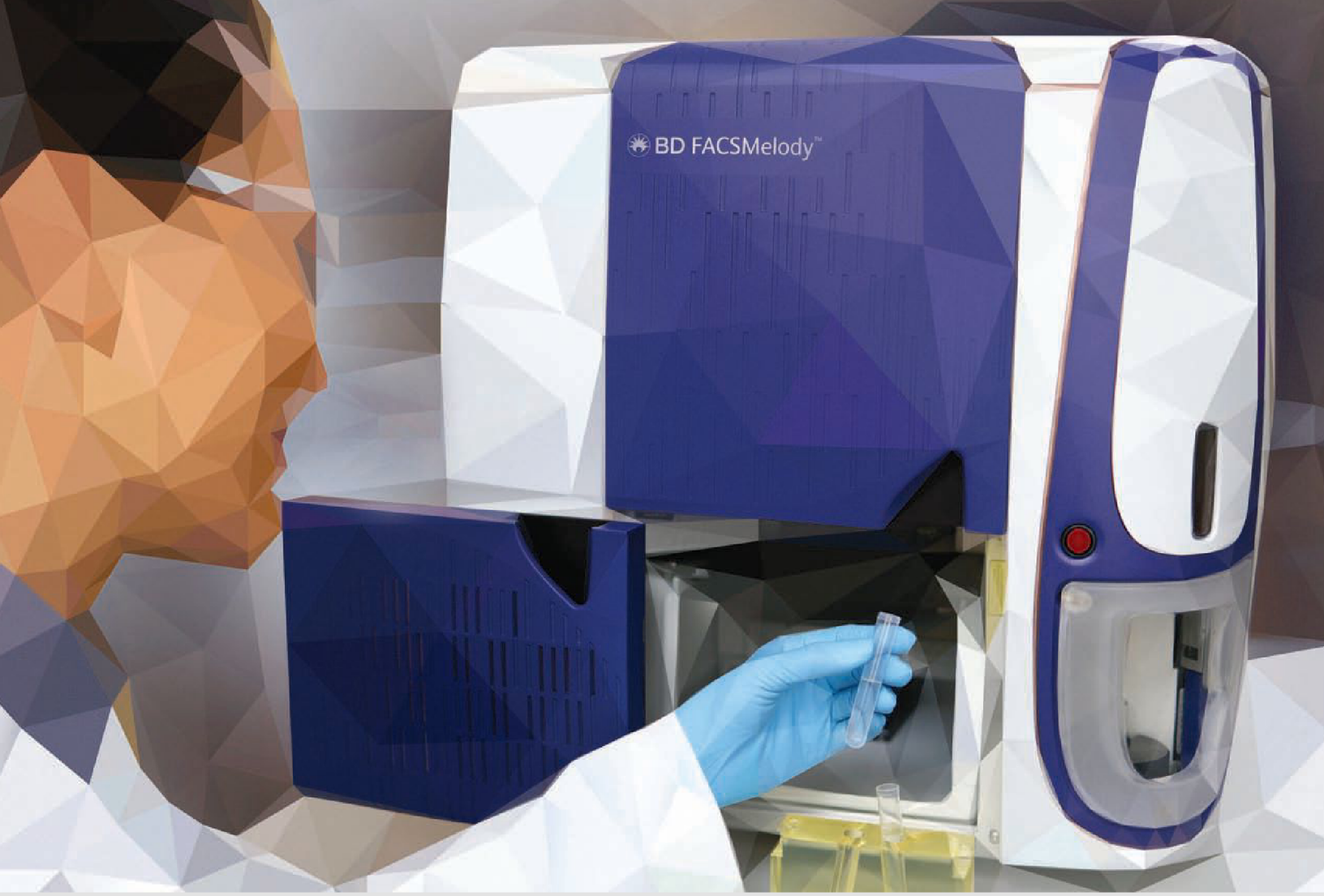
Nature 2.0. The *E. coli* work lays the groundwork for synthesizing whole genomes of larger organisms, including that of humans, an endeavor Church and his colleagues are calling the Genome Project-Write. "The *E. coli* project is not quite complete, but what is clear is that we have done a lot of the hard part. . . . We think this is the flagship experiment for Genome Project-Write in that it shows how you can synthesize big pieces of DNA and then replace them in a chromosome by mainly using phage integrases. The point of the project is to improve technology and to bring down costs," says Church whose group is already at work synthesizing long pieces of human DNA. "The synthesis is quite cheap, about $2,000, but assembly and testing of even a 4-million-base-pair genome is still quite challenging," For Church, synthetic genome applications include virus-resistant agricultural species, as well as human cells for industrial production of human proteins, vaccines, and other therapies. "People who take time to study what the project actually is say, 'Interesting.' Some people imagined a parallel project aiming for parentless babies and that is clearly not what we are doing. We are constructing cell lines."

CHURCH COMMENTS

Waiting for the right moment. "I think most of the failures in the lab are just 'failures so far'—projects that haven't worked yet or that have taken longer than what would be ideal, although I tend not to have particular time lines in mind for projects. I generally don't give up a project, but may put it on the back burner at low priority. Some of these things, like nanopore sequencing, take 25 years before they work well."

Grandfathered in. "My daughter and her family live right next door to me, which is delightful. My granddaughter is 21 months old, which is a really fun age (well, all of her ages are fun). Seeing her grow every day is like having another daughter with a 25-year difference in perspective. It really expands my horizons to see things through such wonderfully alien eyes."

Perfect match. George Church met his wife, Ting Wu, in graduate school at Harvard. "She's a way better scientist than I am. We've worked together on ultra conserved elements, Oligopaints technology, and the Personal Genetics Education Project, aimed at engaging the public on genetics." ■



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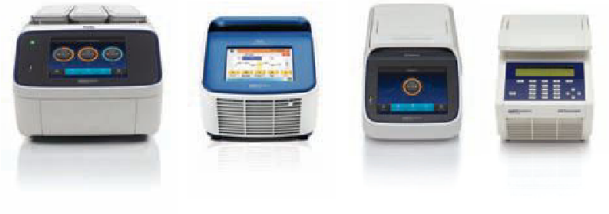

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Lisa Boyette: Catalyzing Cures

Founder and CEO, Curable; Adjunct Assistant Professor, University of Pittsburgh. Age: 35

BY ALISON F. TAKEMURA

In 2002, Lisa Boyette was a first-year medical student at the University of Virginia (UVA) when her 14-year-old brother, Jon, was taken to the emergency room. She rushed to see him. His skin had yellowed with jaundice as his immune system destroyed his red blood cells, a condition called autoimmune hemolytic anemia. "He was so weak and so young," Boyette recalls. It was the first time he had been gravely ill, but it wouldn't be the last.

Meanwhile, Boyette was training for her MD/PhD. She wanted to be a doctor, as well as a scientist who could affect patients' lives through research. But when she couldn't find a project she was passionate about at UVA, she opted to finish her PhD research at the National Institutes of Health with bioengineer Rocky Tuan, now at the University of Pittsburgh. In Tuan's group, Boyette discovered a way to make larger numbers of differentiated bone and cartilage cells from stem cells by using low-oxygen conditions, mimicking the cells' in vivo environment.[1] Her unorthodox path through the MD/PhD program impressed Tuan. "She never gave up," he says.

Boyette supplemented her research with a science policy fellowship investigating new ways of tackling medical problems. In 2009, in collaboration with the Institute of Medicine, she researched case studies on how biomarkers might diagnose chronic disease[2] and helped craft a framework to revamp the national clinical trials system.[3]

In 2009, when Jon was 21, he was diagnosed with primary sclerosing cholangitis (PSC), an autoimmune disease of the bile ducts. His earlier bout with anemia had been a prelude to this more serious condition. Without liver transplants, more than half of patients die within a decade. "I thought, 'Why can't we fix this?'" Boyette says. Because PSC is so rare, affecting only 1 in 10,000 people worldwide, few researchers or pharmaceutical companies have devoted resources to it.

In 2014, Boyette, her brother, and University of Pittsburgh geneticist Dietrich Stephan formed a nonprofit organization to coordinate research on novel therapies for PSC patients. Stephan says Boyette was "incredibly brave" to abandon the security of an academic track. Boyette named the company "SAVE JON." With about $750,000 in revenue so far, the company has attained $15 million in research funding.

Boyette now helps coordinate research by other academic scientists to sequence the exomes of 30 patients and identify potential genetic variants that predispose people to PSC. She has teamed with Garry Nolan of Stanford University to characterize the immune systems of the patients and with Eric Schadt of the Icahn School of Medicine at Mount Sinai to decipher patterns in the data using machine-learning algorithms. Boyette aims to have drugs to treat PSC ready for Phase 2 clinical trials in just five years.

"I think this is *the* new model for how we should approach diseases," Stephan says. Because its approach could ultimately be used to address any disease, SAVE JON was renamed Curable in July.

Through Curable's efforts, Boyette hopes to save her brother. But her company doesn't have to be the one to make the breakthrough, she says. "If somebody else were to wake up tomorrow morning with a cure for PSC, my family would rejoice," Boyette says. "There are plenty of other problems for us to go sink our teeth into."

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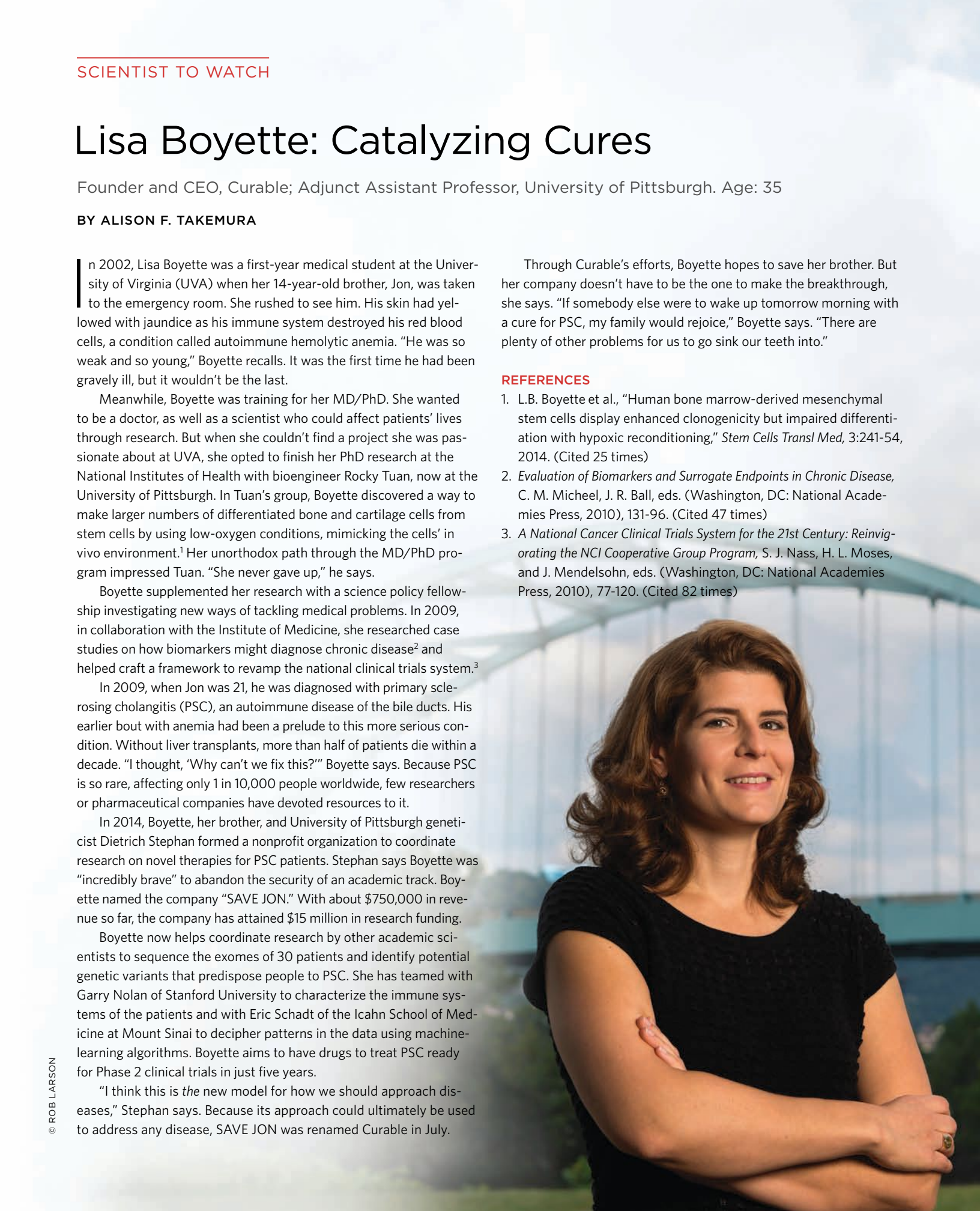

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Mixed Marriages

Using mass spec to understand how proteins interact with small molecules, lipids, and nucleic acids

BY JEFFREY M. PERKEL

Mass spectrometry is today's go-to technology for proteomics research. The technology makes it relatively straightforward to identify and quantify proteins across a range of samples, as well as to detect the posttranslational modifications that so often govern their behavior.

Such studies tend to treat proteins as isolated entities, effectively inventorying them on an organelle, cell, or tissue scale and drawing inferences from the way those tallies change across conditions. But proteins rarely act alone.

Proteins often aggregate into large multiprotein complexes, for one thing. (See "Cracking the Complex," *The Scientist,* November 2015.) They also bind to small molecules, lipids, and nucleic acids.

The Scientist asked researchers who have applied mass spectrometry to a number of such interactions for the lowdown on their techniques.

PROTEIN-DNA

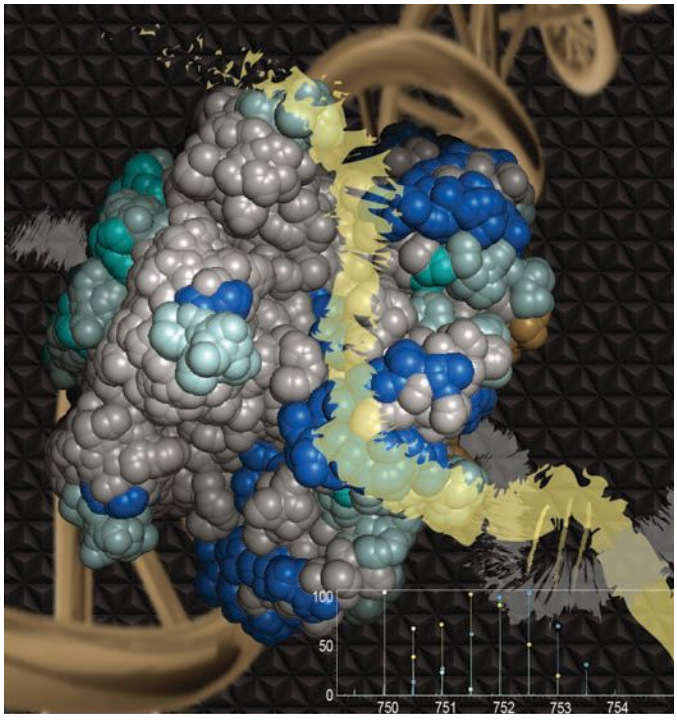
RESEARCHER: Michael Trakselis, Associate Professor, Department of Chemistry & Biochemistry, Baylor University

PROJECT: Mapping protein-DNA interactions in the mini chromosome maintenance helicase (MCM) of the archaeon *Sulfolobus solfataricus* (Sso)

APPROACH: Helicases are ringlike DNA-unwinding enzymes through which one strand of nucleic acid passes, like thread through a needle. But where does the second strand go? Trakselis's team originally attempted to map protein-DNA contacts in SsoMCM complexes using what he calls a "hunt-and-peck–type approach"—mutating specific protein residues one by one and monitoring the impact. But that was tedious, he says. He wanted a global approach that could scan the entire protein sequence simultaneously.

His solution: hydrogen-deuterium exchange (HDX) mass spectrometry. HDX monitors the accessibility of protein residues to the solvent that surrounds them by quantifying changes in the ability of amino acids to exchange protons with their environment over time.

The experiment starts by placing the protein and DNA in deuterated water in the absence of ATP; the lack of molecular energy immobilizes the complex. After waiting up to four hours for exchange to take place, the protein is digested into peptides, which are then sequenced in a mass spectrometer. Protein regions that are bound to DNA will be less solvent-accessible than those that are not, a difference that is marked by the presence or absence of a 1 Da mass shift in the peptide bond (*J Biol Chem*, 291:12467-80, 2016). "If DNA binds to a site, then that amide proton is no longer freely exchangeable," Trakselis explains. By mapping those locations onto the protein's 3-D structure, sites of interaction become clear.



BINDING PATH: For the archaeon *Sulfolobus solfataricus*'s mini chromosome maintenance helicase (SsoMCM), high-resolution mass spectrometry monitors the rate of deuterium uptake for free protein compared with DNA-bound protein. The differences (shades of blue) can then be mapped back onto the protein structure to identify putative binding paths for single-stranded DNA (yellow) on the exterior surface of the SsoMCM helicase.

Even low-end mass spec machines can easily detect such differences, assuming the signal is strong enough. But SsoMCM is built of six identical subunits arranged in a ring. At most, Trakselis says, the DNA would interact with one or two of those. "We needed a high-resolution instrument just to really get above that background of redundancy." So he applied for time on one of the world's most powerful detectors, a 14.5-Tesla Fourier transform ion-cyclotron (FT-ICR) mass spectrometer at Florida State University's National High Magnetic Field Laboratory, which found the project compelling enough to agree to a collaboration.

Using this instrument, Trakselis was able to probe more than 98 percent of the protein sequence in one go. The data suggest that the second DNA strand makes specific contacts with the out-

MICHAEL TRAKSELIS

side of the helicase ring, as opposed to just hanging there like a floppy noodle. "[The helicase is] really grabbing hold of both strands," he says, "but it's doing it in two locations." Now, he hopes to exploit those data to develop new models of enzymatic activity and to identify potential targets of anticancer therapeutics.

A LITTLE ORBITRAP'LL DO YA: FT-ICRs are exceptionally powerful, but also expensive and hard to come by. The biggest and most powerful are housed in dedicated national facilities such as the FSU MagLab and the Pacific Northwest National Laboratory. Trakselis says he only used one because of the unique requirements imposed by SsoMCM. Other helicases, which comprise six different subunits, could likely be solved on more widely available (but still high-end) Thermo Fisher Scientific Orbitrap mass specs, he suggests, as it would be easier to distinguish signal from noise.

PROTEIN-RNA

RESEARCHER: Dirk Ostareck, Professor, Head of Research, Clinic for Surgical Intensive Care and Intermediate Care, University Hospital RWTH Aachen, Germany

PROJECT: Cataloging protein-RNA interactions during macrophage activation

APPROACH: Macrophage activation leads to inflammation, and is tightly regulated on several levels. Among other things, protein binding to key mRNAs can regulate the macrophage's ability to read out specific genetic instructions, including those that produce cytokines. But which proteins perform that role?

To find out, Ostareck and his team used mRNA interactome capture. First described in 2012 by Ostareck's collaborators Matthias Hentze and Alfredo Castello of the European Molecular Biology Laboratory, this method involves cross-linking protein-RNA complexes using ultraviolet light and capturing those complexes that contain polyadenylated mRNAs. The captured material is then treated with proteinase (to isolate RNA) or RNase (to isolate protein) and analyzed by deep sequencing or liquid chromatography–coupled mass spectrometry, respectively (*Mol Cell Proteomics*, 15:2699-714, 2016).

In previous studies, researchers applied this method to cell lines such as HeLa and HEK 293; here, the team applied it to control and bacterial lipopolysaccharide-induced murine macrophages. They identified a total of 402 RNA-binding proteins, which the authors dub the "macrophage RNA interactome."

Comparison of this data set with other published studies identified 32 proteins that appear to be macrophage-specific, Ostareck says, 19 of which had no known link to RNA, including the enzyme P23, a prostaglandin E synthase. P23 binds to mRNA strongly in untreated macrophages, but less so in activated cells. That, Ostareck says, suggests the protein plays a role in mRNA translation control and/or stability regulation—a hypothesis his team is now investigating directly.

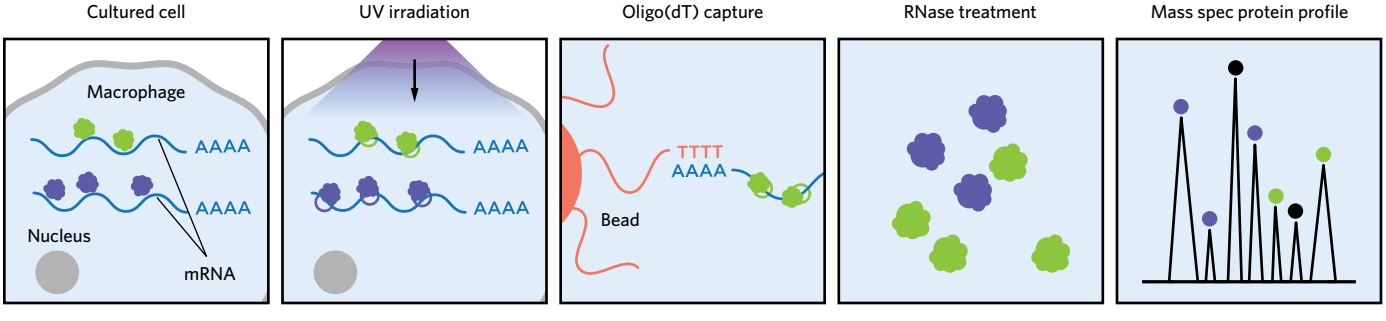
MAPPING DOMAINS & TRANSCRIPTS: There's more than one way to skin a protein-RNA complex, of course. Using RNA immunoprecipitation and deep sequencing, researchers can identify the targets of specific RNA-binding proteins. And using a modification of the interactome approach, called RBDmap, they can map the specific protein domains that actually contact the RNA transcript itself—a strategy Hentze and others recently demonstrated in cardiomyocytes (*Cell Reports*, 16:1456-69, 2016). "This is the next important thing," Ostareck says, "to find both the mRNAs which are bound by the proteins, and especially those mRNAs which are specifically regulated." From there, he says, it may be possible to disrupt this regulation to modulate the inflammatory process itself.

PROTEIN-SMALL MOLECULE

RESEARCHER: Jordan Meier, Investigator and Head of the Epigenetics and Metabolism Section, National Cancer Institute, Frederick, Maryland

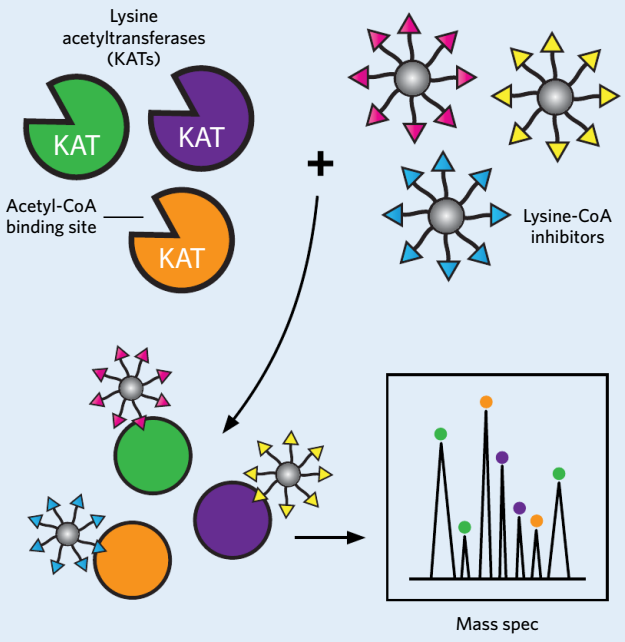
PROJECT: Characterizing metabolic regulation of lysine acetyltransferases—proteins that act as epigenetic regulators

APPROACH: Some labs study metabolism, others study epigenetics; Meier studies both. "Our goal is essentially to understand the underlying biology that connects metabolism with



INTERACTOME CAPTURE: Dirk Ostareck froze mRNA-protein interactions in macrophages with UV light, captured them on oligo(dT)-coupled beads, digested the mRNA, and subjected the proteins to mass spec. The resulting "mRNA interactome" comprised 402 proteins, some of which likely regulate macrophage activation during inflammation.

FIGURE BASED ON A. CASTELLO, WWW.BIOCH.OX.AC.UK/ASPSITE/INDEX.ASP?PAGEID=1141



WHERE METABOLISM MEETS GENE REGULATION: Jordan Meier used bead-bound inhibitors to identify and quantify cellular KATs. By then comparing the affinity of these enzymes for coenzyme-A (CoA) and acetyl-CoA, he could determine which are likely targets of feedback regulation pathways.

epigenetic signaling," he says. Specifically, how do changes in metabolite concentrations influence gene expression?

Fasting, for instance, leads to elevated levels of the small-molecule metabolite coenzyme-A (CoA). CoA is a negative inhibitor of lysine acetyltransferases (KATs), a class of proteins that alter gene expression by "writing" epigenetic marks on histones. "However, because of a lack of methods for looking at [KATs] directly in proteomes, researchers don't really know which acetyltransferases this mechanism is talking to," Meier says. His team developed a chemical proteomics strategy to work that out (*J Am Chem Soc*, 138:6388-91, 2016).

Rather than express and test each enzyme in vitro, Meier's team went fishing. For bait, they used lysine-CoA, a KAT inhibitor that interacts with the enzymes' acetyl-CoA binding site. Conjugation of Lys-CoA to tiny beads enables KATs to be enriched from eukaryotic cell extracts and identified via peptide sequencing on an Orbitrap mass spectrometer.

To assess those proteins' susceptibility to CoA feedback regulation, they compared the enzymes' affinities for either CoA or acetyl-CoA using quantitative Western blotting. They hypothesized that KATs susceptible to feedback inhibition may bind CoA more strongly than acetyl-CoA, Meier explains. One newly discovered enzyme, called NAT10, fit the bill, exhibiting an affinity profile indicative of feedback regulation. "This is really a hypothesis-generating tool," he says.

PROBE DESIGN MATTERS: Meier says chemical proteomics provides a platform that is not limited to KATs. But good probe design is required. Lysine-CoA, he explains, is just a starting point for KAT purification, capturing about 9 of 30 known cellular KATs. By modifying that probe, the researchers recovered an additional 14 enzymes. In the case of KATs, he says, that strategy could facilitate a novel screening strategy. "Could you basically use this approach, where you tether CoA to a peptide that essentially represents a target where we don't know what acetyltransferase modifies it, and now start to pull out candidate enzymes that might catalyze that modification?" Work to answer that question is ongoing.

PROTEIN-LIPID

RESEARCHER: Arthur Laganowsky, Assistant Professor of Chemistry, Center for Infectious and Inflammatory Diseases, Texas A&M University, Houston

PROJECT: Detailing lipid-protein interactions

APPROACH: Generally, proteins are digested into peptides prior to mass spectrometry. Such treatment simplifies analysis, but it also eliminates any possibility of decoding macromolecular complexes. (See "Birds Eye Proteomics," *The Scientist*, July 2014.) "When you chew it up, you really have no idea what you have in the tube, besides the sequence of the protein," Laganowsky explains.

An alternative is native mass spectrometry, which analyzes protein complexes in their intact forms. Laganowsky learned that technique as a postdoc in the University of Oxford laboratory of Carol Robinson, who pioneered the field. But he had to tweak it before he could apply it here, as Laganowsky is interested in understanding the molecular forces that drive protein-lipid interactions. And those are influenced by temperature.

Laganowsky and his team modified a Waters quadrupole–ion mobility–time-of-flight mass spectrometer to precisely control the temperature of the mass spec sample source—which normally operates at room temperature—between about 22 and 42 °C. They then solubilized the *E. coli* ammonia channel, AmtB—a model integral membrane protein—in detergent, titrated in a series of phospholipid ligands over a range of temperatures, and measured the complex size using mass spec. From that, they calculated the thermodynamic properties of each lipid-protein interaction, discovering that different lipids bind in fundamentally different ways (*J Am Chem Soc*, 138:4346-49, 2016). "That was quite surprising, that you could see these different signatures," he says. And applying the same approach to a specific AmtB mutant altered those signatures in revealing ways. "Each of these lipids have different responses of entropy and enthalpy."

MACHINING NOT REQUIRED: Mass-spec customization is often expensive and technically difficult. Not so with Laganowsky's temperature control system. The original prototype involved basically a temperature controller, a few CPU fans and power supplies, cardboard, and tape. "Some of my colleagues laugh when I tell them this," he admits, "but it's relatively cheap to do and it requires no modification of the instrument." And, he adds, there's no reason the same approach wouldn't work for other classes of macromolecules, too. ■

ADAPTED FROM D.C. MONTGOMERY ET AL., *J AM CHEM SOC*, 138:6388-91, 2016.

Counting Copy Numbers

As the importance of genomic copy number variations for health and disease becomes clearer, researchers are creating new ways to detect these changes in the genome.

BY SARAH C.P. WILLIAMS

A decade ago, scientists studying the human genome found 1,447 copy number variable regions, covering a whopping 12 percent of the genome (*Nature*, 444:444-54, 2006). Ranging in size from 1 kilobase to many megabases, the number of repetitive DNA sequences scattered throughout the human genome can expand and contract like an accordion as cells divide. Extra—or too few—copies of these repeats, known as copy number variations (CNVs), can explain inherited diseases or, when the copy number change occurs sporadically in somatic cells, can result in cancer. Today, a growing number of scientists are making links between CNVs, health, and disease.

But measuring CNVs in cells from an individual can be tricky. For a number of years, researchers relied on fluorescently tagged microarray probes that attached to sections of genes; locations where the probes fluoresced more or less brightly than in an average genome suggested duplications or deletions of repeats within the CNV region, but the resolution was generally low. Throughout the early 2000s, researchers moved toward using higher-resolution microarrays to detect CNVs, and commercial kits became available that provided the probes needed for these assays. More recently, the advent of high-throughput genome sequencing has offered a new way to detect and quantify, or "call," CNVs.

"This seems to be the next wave in CNV calling," says computational biologist Dan Levy of Cold Spring Harbor Laboratory. "Things are moving from microarray to sequencing-based approaches."

But sifting through an entire genome to find changes to CNVs is no easy task either, whether you're starting from a whole genome or an exome.

"Exome sequencing was optimized to detect small things, like single-nucleotide variants or deletions, not for CNVs," says Yufeng Shen, a computational biologist at Columbia University. "So the data is quite noisy for detecting CNVs."

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To sort through this noise, researchers are developing new computational tools, each of which takes a slightly different approach to finding CNVs. *The Scientist* spoke with the creators of four recently debuted open-source tools about what distinguishes their approaches and when you should consider using their software.

CANOES: FINDING RARE, SMALL CNV DELETIONS

THE PROBLEM: Shen was studying the genetics of heart disease when he decided that the existing tools weren't working for him. He wanted to detect small, rare copy deletions in CNV regions that might be related to heart-disease predisposition. Using whole-genome sequencing was expensive, but he didn't like the messiness of the data that came from cheaper exome sequences.

"Exome sequences are biased toward a reference genome," he points out. "You're creating probes based on what you already know you want to sequence."

Many methods that analyze exome data to detect CNVs rely solely on read depth—essentially, the number of times a section of the genome is read during sequencing. In collecting sequence data, more reads means a more accurate sequence. But in CNV regions, that number of reads, or depth, is also correlated with the number of repeats. More reads usually means an expanded CNV region with extra copy numbers, while a lower depth of coverage than usual can indicate deleted repeats in the CNV region. Shen, though, thought that the inherent bias and noisiness in exome sequences wasn't being

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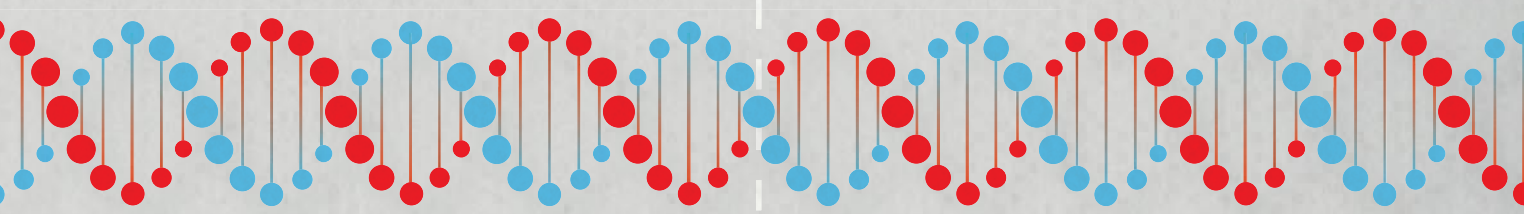
GENOME EDITING

With CRISPR/Cas9 – An Introduction



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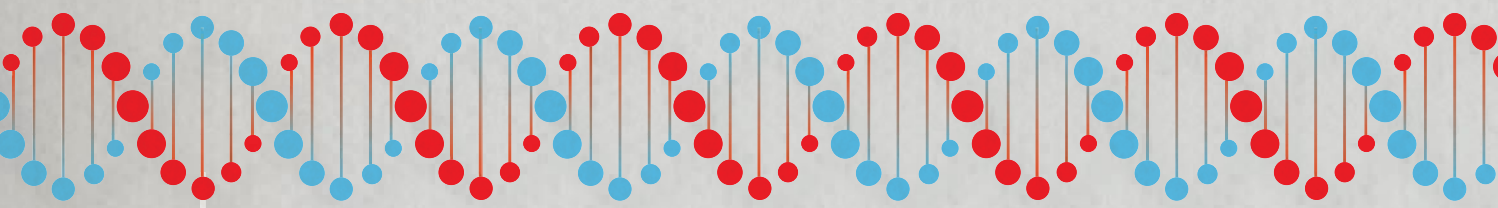
GENOME EDITING

With CRISPR/Cas9 – An Introduction

Discovered nearly 30 years ago, the CRISPR/Cas9 system has gone from a form of bacterial innate immunity to a game-changing genome editing technology, completely revolutionizing molecular biology within the past five years. Learn more about the mechanism, protocol, and groundwork that have brought this method to laboratories across the globe.



1987
"Unusual structure" in *E. coli iap* gene identified by Ishino et al. [1]

2000
Short Regularly Spaced Repeats (SRSR) proposed [2]

2002
CRISPR acronym first used to define Cas gene clusters [3]

2005
Spacer sequences matched to foreign DNA [4-6]

2005
First evidence of Cas protein [7]

2006
Adaptive immunity f
of CRISPR proposed

2001 2003 2005 2007

BASIC CRISPR WORKFLOW:

1. Design sgRNA(s) and/or donor DNA fragment(s)
2. Introduce Cas9 and sgRNA into your cells

CRISPR GLOSSARY:

Cas9 (n.)
CRISPR-associated protein #9

CRISPR (n., adj.)
Clustered, regularly-interspaced, short palindromic repeat

crRNA (n.)
CRISPR RNAs

DSB (n.)
Double-stranded break

2008

2010

2012

2014

2007
First experimental evidence of CRISPR adaptive immunity [9]

2008
The term "protospacer"coined [13]

2008
CRISPR cuts DNA target [12]

2009
First use of "protospacer-adjacent motif" (PAM) terminology [16]

2010
Mechanism for self vs. non-self identified [18, 19]

2011
Evidence of tracrRNA complexing with crRNA and Cas9 [21]

2012
Single-protein requirement for type II Cas9 revealed [23, 24]

2013
CRISPR/Cas9 used in human cells [25-27]

2013
CRISPR/Cas9 used in plant cells [28, 29]

2014
PAMs revealed as key driver of target interrogation [30]

2014
PAM/Cas9 interaction assists in local strand separation & R-loop formation [31]

2014
Direct delivery of Cas9 & sgRNA to cells [32]

2007
CRISPRdb becomes available [10]

2008
crRNA processing pathway defined [14, 15]

2007
CRISPR/Cas9 secondary structure classification [11]

2009
RNA-guided RNA cleavage described [17]

2010
crRNA guides Cas9 cleavage of DNA via DSBs [20]

2011
Type II CRISPR genes expressed in other organisms [22]

2009

2011

2013

2015

3 Induce Cas9 and sgRNA expression; add donor DNA

4 Allow cells to recover

5 Analyze cells for gene-editing events, either individually or in pools

dsDNA (n.) Double-stranded DNA

GMO (n.) Genetically modified organism

HDR (n.) Homology-directed repair

HNH (n.) A nuclease domain that cuts the target strand

Indel (n.) Insertion or deletion

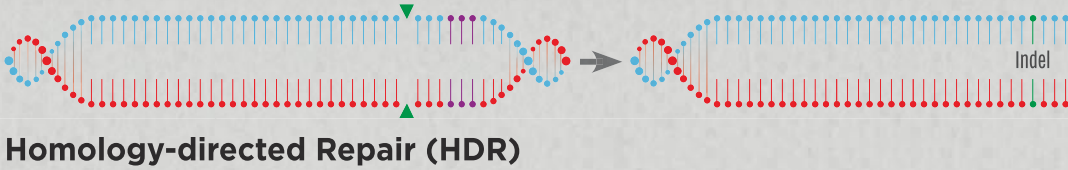
NHEJ (v.) Nonhomologous end joining

NUC (n.) Nuclease lobe of Cas9

REPAIR MECHANISMS:

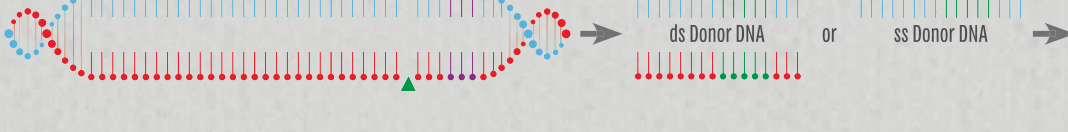
Nonhomologous End Joining (NHEJ)

Error prone · Random mutation of 1-10 indels · High-frequency, sgRNA-dependent event



Homology-directed Repair (HDR)

dsDNA or ssDNA donor DNA required · Either corrects known mutations, adds foreign DNA, or replaces defined fragm

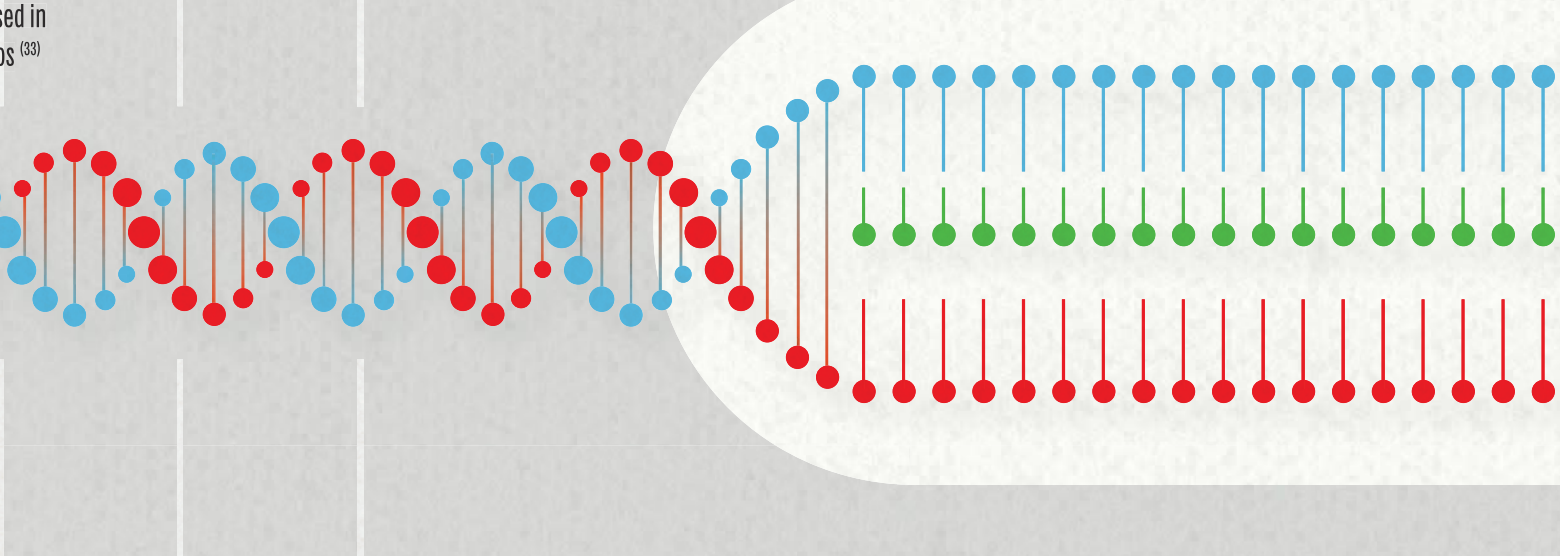


2016

USDA determines edited crops will not be regulated as GMOs [35]

LS

sed in
os [33]



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$$\text{Fraction cleaved} = \frac{\frac{\text{Concentration of Fragment 1} + \text{Concentration of Fragment 2}}{2}}{\left(\frac{\text{Concentration of Fragment 1} + \text{Concentration of Fragment 2}}{2}\right) + \text{Concentration of Intial Amplicon}}$$

$$\text{Fraction Mutated} = 1 - (1 - \text{Fraction Cleaved})^{0.5}$$

LS

ng prompts a discussion [34]

2016

NIH approves first human trial [36]

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PAM (n.)
Protospacer-adjacent motif

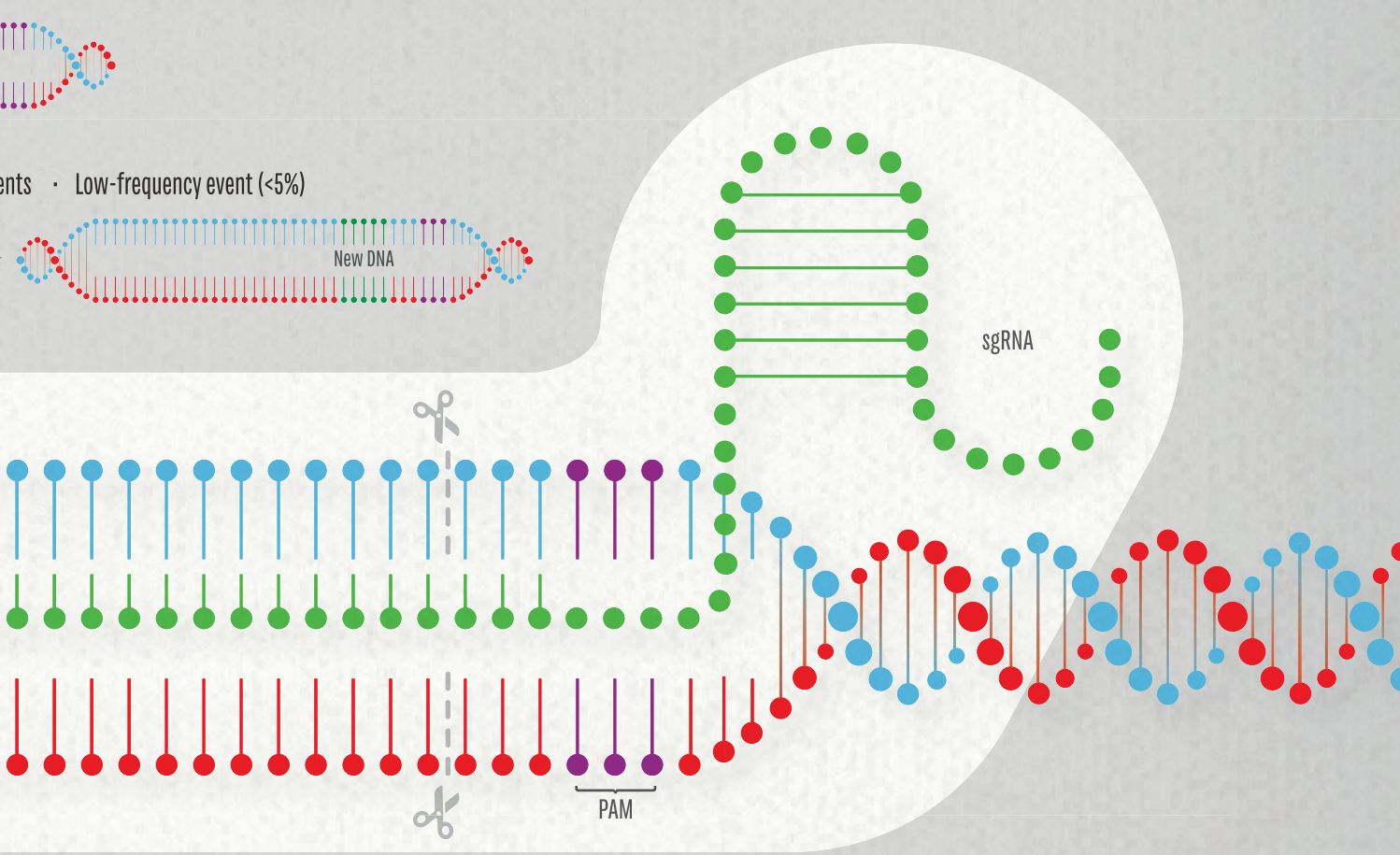
REC (n., adj.)
Recognition lobe of Cas9

RuvC (n.)
A nuclease domain that cuts the noncomplementary strand

sgRNA (n.)
Single guide RNA

ssDNA (n.)
Single-stranded DNA

tracrRNA (n.)
Trans-activating crRNA



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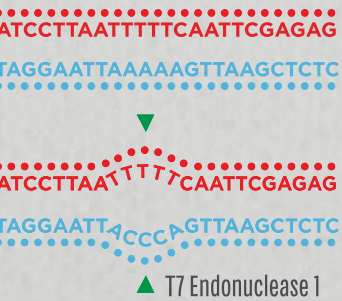

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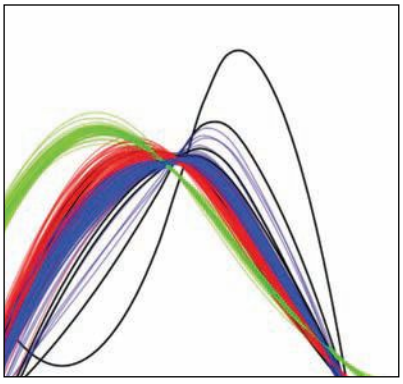
CRISPR is becoming the main procedure to knock-in or knock-out genes or alter genetic sequences. Due to its simplicity, multiplexing capability and reagent availability, researchers are exploring the limits of its capabilities in model systems and for clinical applications. Efficient screening and detection of gene editing events is critical to successfully generating edited cell lines or organisms.

Advanced Analytical Technologies, Inc. is a world leader in parallel capillary electrophoresis instruments for automating genomics discovery. Our award winning instrument, the Fragment Analyzer™ Automated CE System, is the premier instrument for analyzing nucleic acid fragments and smears and has become the industry standard for most genomics applications. Created to streamline laboratory workflow and decrease time to results, the Fragment Analyzer can:

- Reliably quantify and qualify DNA fragments, NGS libraries, genomic DNA, total RNA, and small RNA.
- Analyze gene editing events generated through CRISPR/Cas9 mutagenesis.
- Accurately size all gene editing events, in both pooled and individual samples.
- Assess the relative quantity to determine the mutation frequency percentage and zygosity.
- Generate results with minimal effort through the aid of a specially designed CRISPR software package.

The CRISPR experimental design dictates whether a few or many cells with edits are generated from a population of pooled cells. Editing is dependent on many factors including: the expression or delivery of the guide RNA(s)/Cas9 complex, delivery or expression of donor DNA, effectiveness of the guide RNA(s) and efficiency of repair mechanisms. These factors all have an impact on gene editing mutation frequency yield. The Fragment Analyzer can be used to screen the pooled cell populations post-editing. Measuring the overall effectiveness of the experimental strategy helps determine how many cells need to be evaluated in order to choose and propagate cells with the desired modifications. To assist in winnowing the cells with known edits, the Fragment Analyzer can also be used to identify which individual cells have edits at one or more than one of the alleles, essentially zygosity determination. Being able to identify monoallelic from diallelic events in diploid organisms greatly reduces the amount and costs associated with sequencing to identify the organism with the desired nucleotide changes.

As CRISPR becomes the premier gene editing procedure, doesn't it make sense to couple it with the premier instrument for fragment analysis? Find out more at www.aati-us.com/crispr



INHERENT BIAS: Different sequencing samples (grouped into red, blue, and green) show different relationships between read count and GC base content. CANOES selects samples that are most similar (black lines) to use as reference genomes.

considered appropriately—at a statistical level—by many of the CNV calling programs. So he created CANOES.

THE SOLUTION: By virtue of the inherent variability in sequencing, the read depth on every area of the exome will never be identical. Many CNV analysis algorithms use a Gaussian bell curve to represent the normal distribution of depth of coverage. But Shen's CANOES program instead relies on a negative binomial distribution, a curve that assumes more regions will have a low depth of coverage, even without changes to CNVs (*Nucleic Acids Res*, doi:10.1093/nar/gku345, 2014). Changing the curve may seem like a small modification, but Shen says it makes all the difference in being able to use messy exome data to find small changes of just a few repeats to CNV regions.

"We're acknowledging that the data is very noisy and assuming the CNV of interest is relatively rare in the data set," he says.

The other key to CANOES is that if you have a particular exome of interest, such as one from a person with heart disease, that you're comparing with a bunch of controls, it doesn't automatically use all the controls. Instead, Shen finds reference exomes that have similar read depth to the exome of interest in most areas. That helps avoid false positives popping up simply because of the normal variation in reads.

"What we do is ID samples that, globally, are very similar to the sample of interest," he says.

HOW AND WHEN TO USE: CANOES is available for free (www.columbia.edu/~ys2411/canoes), but Shen warns that it's not for the beginner.

"In order to use it, you have to have a good understanding of the statistical problem of calling CNVs and also know that you'll have to pick the right reference samples," he says.

Since the program shines at detecting deletions, he suggests using it at the same time as other methods that might be better at picking up on duplications.

"I'd tell people to choose three tools that all use models so that they are complementary to each other," he says.

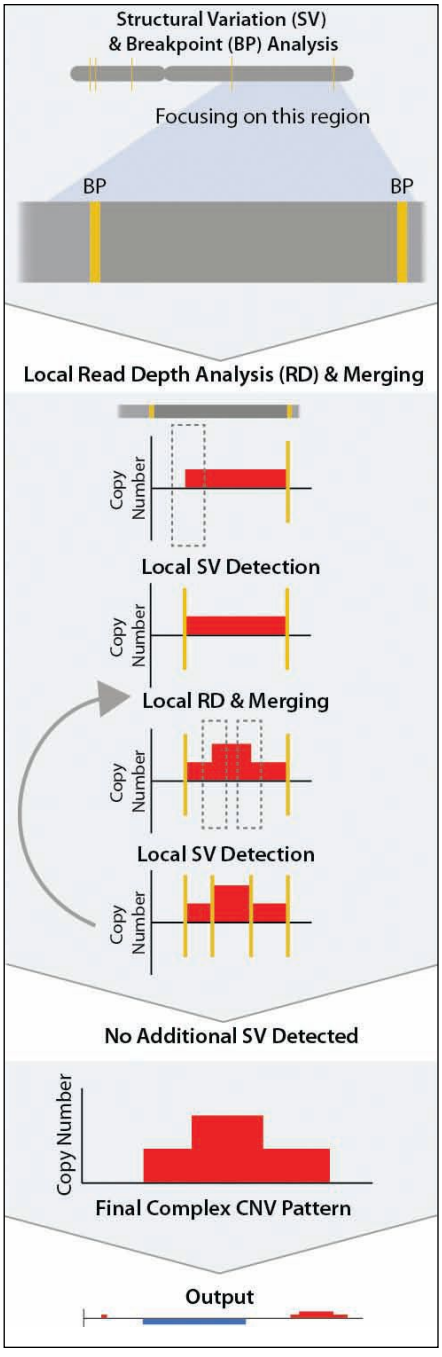
CONSERTING: MOVING BEYOND READ DEPTH FOR CANCER CNVS

THE PROBLEM: In 2010, computational biologist Jinghui Zhang of St. Jude Children's Research Hospital in Memphis, Tennessee, was collaborating with colleagues to study the genomes of pediatric cancers. Along with gene mutations, she wanted to find copy number variations that might be linked to some tumors.

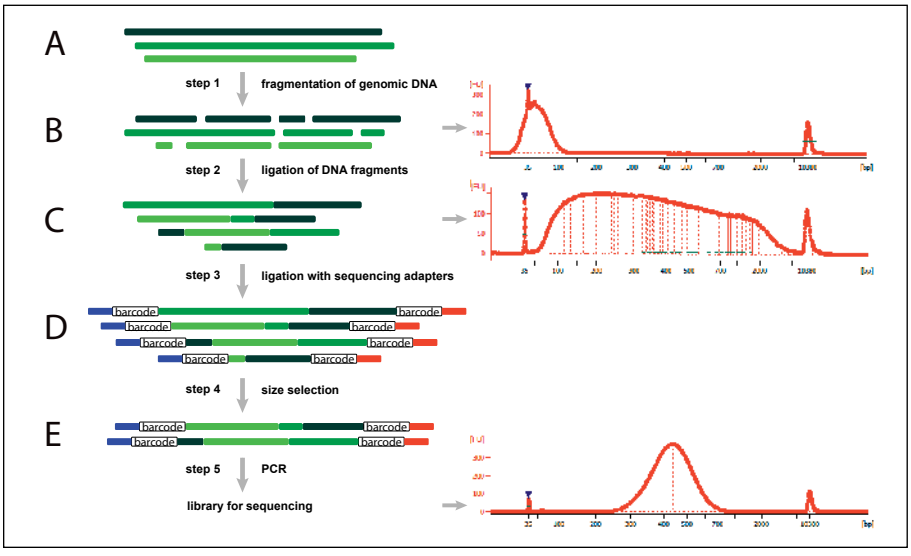
"We knew there were other tools available, and we kept running the tools on our data and trying to compare what we got, but to our surprise, the matches between all the tools were very poor," says Zhang. In retrospect, she says, the tools were developed to find germline CNVs, not somatic changes that arise in cancers. "Tumor analysis is quite different from germline copy number analysis," she says. "The size of somatic CNVs is more variable, and they can be present in only some cells of a tumor, requiring greater sensitivity to detect." So Zhang spearheaded her own approach.

THE SOLUTION: Copy Number Segmentation by Regression Tree in Next-Generation Sequencing (CONSERTING) uses more than just read depth to find CNVs (*Nat Methods*, 12:527-30, 2015). The algorithm also combs the genome for structural variations, such as breakpoints—sequence locations known to be especially sensitive to damage. If two sequences that are normally adjacent are farther apart than usual,

STRUCTURALLY SPEAKING: In CONSERTING, an iterative analysis of both read depth and structural variations (SVs) reveals CNVs in cancer cells. Structural analysis helps identify breakpoints that could signal places where CNVs have been gained or lost.



COURTESY YUFENG SHEN; COURTESY JINGHUI ZHANG



GENOME RESHUFFLE: This schematic of the SMASH method illustrates how three molecules of DNA are fragmented, rearranged, and tagged with barcodes for PCR amplification and sequencing. The size of the fragments throughout the process is shown on the right.

that points toward increased copy numbers; if two areas normally spaced apart are now close, that hints at a deletion.

"The structural variation support may not be important if you're looking at a long deletion," says Zhang. "But some of these CNV changes are very small and focal, and you might not have statistical power with just read-depth changes."

In cancer, she says, this is even truer than in germline cells, because tumor cells may harbor diverse mutations. And if only some of the cells in a tumor have a copy number variation, the read depth in that area of the genome may not be obviously different.

HOW AND WHEN TO USE: CONSERTING was developed to compare cancer cells with healthy cells from the same patient. But Zhang says it also could be used in germline samples.

The program is available open-access, but "it's not easy for most scientists to use," Zhang says. "We're looking into whether we can create a graphical interface so a regular bench scientist who doesn't have bioinformatics expertise can just upload their data."

SAVING MONEY BY SMASHING THE GENOME

THE PROBLEM: Dan Levy wanted an approach to detecting CNVs that would rival the accuracy of whole-genome sequencing without the cost. "One of the things we're always trying to do is make things cheaper," he says. "That allows much larger studies, or you can make clinical products that are more reasonably priced."

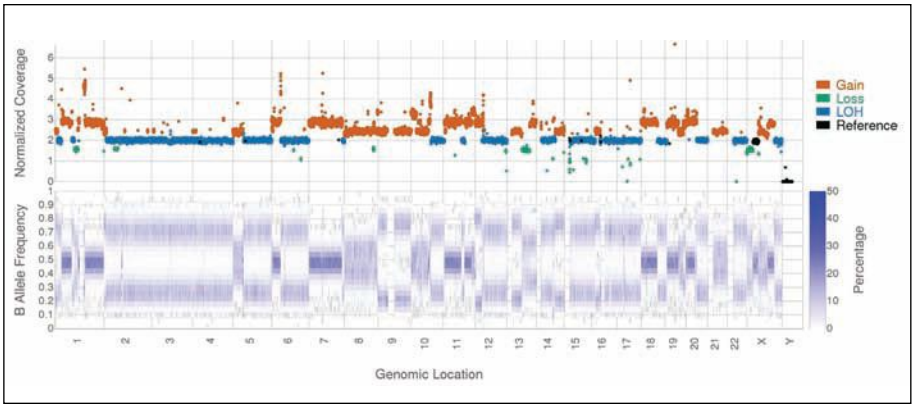
Typically, Levy says, researchers try to drive down the price of genome sequencing by increasing the length of the reads. But when you're looking for CNVs, long reads don't help, because "what you're looking for is an excess of [similar] reads coming from one area of the genome."

Levy realized that all he needed from each read of the genome was enough information to tell whether it belonged in a region. That gave him an idea for how to lower the cost of finding CNVs.

THE SOLUTION: To detect CNVs using the Short Multiply Aggregated Sequence Homologies (SMASH) method, the genome is first mapped using read lengths of about 150 base pairs, the length that currently gives the lowest cost per base. Levy's approach then creates random fragments from those reads, each only 35 to 40 base pairs long. Those fragments are joined together into lengths suitable for creating a library—upwards of 300 base pairs—and tagged. Then, the SMASH software uses that library to generate read counts. Each original read length is used more than once, because of fragmentation (*Genome Res*, 26:844-51, 2016).

"You use long reads to save money, but you use them efficiently," says Levy. "Instead of finding two things from two reads, we fragment and mix up those reads to get four or five mappings."

HOW AND WHEN TO USE: Levy says SMASH should work for most applications—germline or somatic CNVs. "The hope is to get some of these CNV tools into clinical applications," he says. Cheap CNV analysis could be useful for both tumor profiling and prenatal screening, he points out.



CNVs ACROSS A GENOME: Data generated by Canvas are graphed to show CNVs across an entire breast cancer genome. Red and green circles on the upper plot indicate areas with gains and losses in CNVs. The lower plot shows allele frequencies; regions with standard variation have allele frequencies around 0.5.

The approach is relatively easy for other labs to pick up on, Levy adds. "It's all using stuff that's widely available."

CANVAS: AN ALL-IN-ONE TOOL

THE PROBLEM: Eric Roller, a bioinformatics scientist at Illumina, wanted a CNV tool that could help Illumina's broad customer base—researchers who study everything from inherited diseases to cancer using a wide range of data. No existing approaches, he says, met this one-size-fits-all requirement.

"CNVs can vary dramatically in size and copy number due to the different underlying biological mechanisms involved," says Roller. "Extensive CNV heterogeneity means that many tools were designed to detect only particular kinds of variants," he adds. Many existing tools can either look for CNVs in the germline or in somatic cells but not both, or only use whole-genome data or exome data but not both. And as users have admitted, these customized workflows can be daunting for a beginner, requiring manual inputs of certain control data values and tweaks to the algorithms for each project.

So Roller and his colleagues at Illumina took on these challenges. The result: a program called Canvas.

THE SOLUTION: While the software relies on the relatively common approach of measuring read coverage and allele frequency, where it stands apart is the easy-to-use interface and the machine-learning algorithms that automatically compute things such as coverage bins (collections of similar DNA fragments) so researchers don't have to do manual calculations. "An analysis can be started with a single command," says Roller.

To test Canvas's capabilities, Roller and his colleagues searched for CNVs in a range of data sets, including breast cancer cell lines and previously sequenced genomes. Canvas came out with an 87 percent accuracy score and a 77 percent precision rating when used to analyze somatic cell lines—better in both ways than three older free tools the researchers compared it with. For germline and exome sequences, the program also boasted high accuracy and precision ratings. Moreover, it gave results 2.5 times faster than the next fastest method. The results were reported in *Bioinformatics* in March 2016 (doi:10.1093/bioinformatics/btw163).

HOW AND WHEN TO USE: "Canvas is a single software tool that can analyze both targeted and whole genome sequencing data from both tumor and germline samples," says Roller.

The free, open-source software, available from GitHub, runs on Linux or Windows. Roller says he especially recommends it for comparing tumor cells with somatic cells. "This is a case where the data makes copy number calling challenging, due to noisy signal and potentially extensive genome rearrangements," he points out. "It's also an important application, as CNVs can be key markers or drivers of cancer." ■

TS Webinars

COMING**SOON** | Combatting Zika Virus with Synthetic Biology and Genome Editing

The wide adoption of synthetic biology and genome-editing technologies has enabled a heretofore unprecedented response to the appearance of Zika virus in the Americas. In concert, these technologies have led to several advances in the study, diagnosis, and prevention of the mosquito-borne virus. To explore the union of urgency and collaboration that has typified the rapid response, *The Scientist* is bringing together a panel of experts to share their research into understanding and combatting Zika virus, and to explore the lessons learned. Attendees will have an opportunity to interact with the experts, ask questions, and seek advice on topics related to their research.

KEITH PARDEE, PhD
Assistant Professor
Leslie Dan Faculty of Pharmacy
University of Toronto

ZACHARY N. ADELMAN, PhD
Associate Professor
Department of Entomology
Texas A&M University

WEDNESDAY, OCTOBER 12, 2016
2:30–4:00 PM EDT

REGISTER NOW!
www.the-scientist.com/combattingzika
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- Disease detection with paper-based diagnostic tests
- Using gene drives to selectively eradicate vector species

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COMING**SOON** | Cellular Signaling in Metabolic Disease – Dysregulation to Disorder

Metabolic pathways represent the beautifully orchestrated interplay between cellular systems, working in concert to achieve a shared goal, but – despite their power when finely tuned – they can rapidly fall out of sync, giving rise to disease. To better understand the genesis of these diseases and, ultimately, how to reverse or prevent them, researchers are interrogating the integral cell-signaling pathways and assessing their defects. *The Scientist* is bringing together a panel of experts to discuss the methods they use for isolating and investigating these multifaceted pathways, and to share the findings of their latest research. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

NATHAN COUSSENS, PhD
Senior Research Scientist, Biology
Division of Pre-Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health

BEATRICE HAIMOVICH, PhD
Associate Professor of Surgery
RW Johnson Medical School
Rutgers University

TUESDAY, OCTOBER 18, 2016
2:30–4:00 PM EDT

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TOPICS TO BE COVERED:

- The finely tuned cell-signaling pathways of metabolic processes
- Disorders and diseases caused by disruption of metabolic signaling

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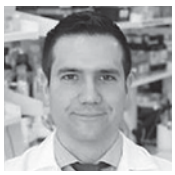
COMING**SOON** | Neurodegenerative Diseases: Peering in on Protein Interactions

As the population ages, neurodegenerative diseases are becoming increasingly prevalent, bringing with them increased health-care costs, caretaker burden, and an urgent research need. Research into the mechanisms of the most prevalent neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, is ongoing, but the molecular underpinnings are still poorly defined. New methods are enabling the direct analysis of protein-protein interactions, and these interactions are painting a clearer picture of the diseases' mechanisms. *The Scientist* is bringing together a panel of experts to share their experience bringing these methods to bear on complex diseases. Attendees will have an opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.



SALVADOR SIERRA-SAN NICOLÁS, MD, PhD
Postdoctoral Fellow
Department of Pharmacological Sciences
Icahn School of Medicine at Mount Sinai

SOPHIA SCHEDIN-WEISS, PhD
Senior Researcher
Center for Alzheimer Disease
Department of Neurobiology, Care Sciences and Society
Karolinska Institutet

WEDNESDAY, OCTOBER 26, 2016
2:30–4:00 PM EDT

REGISTER NOW!
www.the-scientist.com/neurodegenerationpla
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- Using proximity-ligation assays to tease out protein interactions in neurodegenerative disease
- Solving the mysteries of Alzheimer's and Parkinson's diseases

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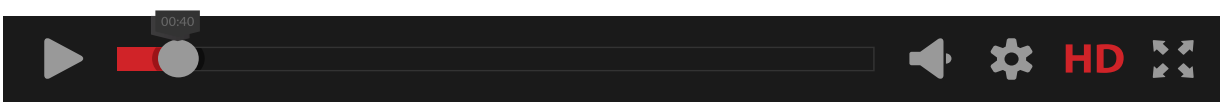
ON**DEMAND** | Biomarker Breakthroughs: Leveraging Liquid Biopsies to Unlock Biological Mysteries

Liquid biopsies are an attractive, minimally invasive method for the detection, evaluation, and monitoring of cancer development and progression. Innovative technologies and strategies exist for profiling samples of cell-free DNA (cfDNA) and DNA from circulating tumor cells (ctDNA). However, technical challenges persist in assay sensitivity and specificity. To explore and illuminate the application of digital PCR for the detection and quantification of biomarkers from liquid biopsies, *The Scientist* brings together a panel of experts to discuss strategies for detecting and quantitatively analyzing mutations in cfDNA and ctDNA and the application of this information to profile disease progression and treatment.



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Molecular Oncology Team
Institute of Cancer Research, UK

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Post-doctoral Researcher
Department of Bioengineering
Stanford University

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Pharma for Furry Friends

Companies focused on adapting human therapies for dogs, cats, and horses are bringing a diverse array of products to the pet medicine market.

BY JENNY ROOD

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Around Christmas 2010, University of Minnesota researcher Mark Suckow received terrible news: his family's twelve-and-a-half-year-old Labrador retriever, Sadie, had squamous cell carcinoma that the vet deemed fatal. But Suckow's children refused to accept that outcome—their father, after all, was both a trained veterinarian and an expert on tissue-based cancer vaccines. Couldn't he make one for Sadie? Although his research was in rodent models, he agreed to give it a try. Using a harvested bit of Sadie's tumor, he created a vaccine by breaking the tumor down into individual cells, inactivating them with a fixative, and mixing them with a bulking agent. Within a couple of weeks—soon after Sadie was injected with the second dose—the tumor stopped spreading and began to shrink. Sadie survived nearly three more years, passing away in 2013 from unrelated causes.

The success inspired Suckow and his student Ashley Kalinauskas to form Torigen to commercialize veterinary cancer vaccines. The company now sells its tissue-based Vetivax technology for dogs, cats, and horses, and has treated more than 70 animals so far without adverse effects, Suckow says. Because the procedure involves injecting tissue back into the same animal it came from, the US Department of Agriculture (USDA)—which is responsible for evaluating animal biologics such as antibodies and vaccines—has cleared the vaccine without requiring a regulatory approval process.

Torigen is just one of many new companies that are delivering therapies to our animal companions. In 2012, the fledgling field experienced a watershed moment when Pfizer spun out its animal health division as Zoetis. "Zoetis's IPO spurred [investor] interest and helped create an ecosystem for smaller companies to raise capital," says Mark Heffernan, CEO of Ireland-based NexVet, which focuses on monoclonal antibody therapies for pets. Zoetis remains a big player in the space, delivering pharmaceuticals for both livestock and companion animals to treat conditions ranging from infectious disease to pain. This year, just in time for the bang of the Fourth of July fireworks, the company released Sileo, a low-dose sedative to address canine noise aversion.

At the other end of the spectrum from the large and diverse Zoetis are smaller companies such as Torigen, built around individual types of therapies, and companies of intermediate size that have built a portfolio out of a variety of products originally developed for people. The result is a diverse and growing industry that continues to churn out new therapies for our pets. As of July, the US Food and Drug Administration (FDA) had already approved six new treatments this year for dogs alone—the same total number approved for both dogs and cats in both 2015 and 2014. The USDA also approved two pet biologics in 2015. And Dorothy Brown, director of the University of Pennsylvania's Veterinary Clinical Investigation Center, has noticed that companies originally aiming for human regulatory approval are increasingly interested in developing compounds for pets.

"A [pet therapeutic] industry has been established," says Heffernan. "Now it's up to us as the early-stage, leading companies to deliver."

From humans to pets . . .

Because it can be financially risky to develop a treatment—for animals or humans—from scratch, many pet therapeutics companies are licensing, tweaking, and testing drugs and antibodies that have already been at least partially trialed for human use. "We like to say that we

don't develop any drugs for pets that have not already been tested in people," says Richard Chin, CEO of northern California-based KindredBio, which develops small molecule drugs as well as biologics for cats, dogs, and horses.

At Zoetis, researchers take inspiration from projects in Pfizer's human health division. In 2013, for example, the FDA approved a Janus kinase inhibitor for canine atopic dermatitis. This pet med was derived from a compound for treating human rheumatoid arthritis that blocks the cytokine signaling responsible for inflammatory reactions. Andrea Gonzales, Zoetis's lead research scientist in dermatology, and her team confirmed the role of cytokine signaling in cell-based and animal models of atopic dermatitis, then tweaked the Pfizer compound to target the appropriate cytokines in dogs (*Vet Dermatol*, 23, Suppl. 1:2-104, 2012). The result was Apoquel, which stopped the itch within hours of treatment and was so successful that it was fairly quickly on back order, says Lisa Troutman, a supervisory veterinarian at the FDA Center for Veterinary Medicine (CVM). When Gonzales and her team sought to understand why Apoquel acted so quickly, they identified a specific target, cytokine IL-31, which they found in the blood of dogs with atopic dermatitis (*Vet Dermatol*, 24:48-e12, 2013). Based on this finding, they developed lokivetmab, a monoclonal anti-IL-31 antibody that was conditionally licensed by the USDA in 2015 for use in dogs.

Other companies look for drugs that failed in people but might work in pets. For example, VetDC licensed its lymphoma treatment Tanovea from Gilead after the compound caused unexpected side effects in human patients. In dogs—200,000 of which are afflicted by the disease in the U.S. each year—a combination of Tanovea and doxorubicin was as effective as a four-drug chemo cocktail, even when administered in half as many visits. By leveraging Gilead's prior research—including the development of the compound and its manufacturing pipeline as well as preclinical trials in dogs—VetDC hopes to reduce the final cost to the consumer. Cost is an important consideration in a market where health insurance is rare. "We don't have Peticare and Peticaid," says Steven St. Peter, CEO of Aratana Therapeutics, which has used the licensing strategy to gain approval of both biologics and small molecules for a variety of canine conditions.

Aratana's most recently FDA-approved product, Entyce, combats canine loss of appetite, which affects one in eight dogs and is the primary reason dogs are euthanized, St. Peter says. Entyce is a ghrelin receptor agonist in development for appetite-related issues in people by the Pfizer spin-off RaQualia of Japan. In preclinical studies, dogs given the drug gained weight, and 80 percent of vets want to stock the product when it's available in February 2017, St. Peter says. While pet therapeutics companies must demonstrate safety and effectiveness to gain FDA approval for a drug, this requires only a single randomized controlled trial, equivalent to a human Phase 3 trial. (See "Regulating Animal Therapeutics" on page 72.) KindredBio has developed a similar therapy for cats: a repurposed antidepressant, mirtazapine, that caused weight gain as a side effect in people. Kindred's reformulation, currently under FDA review, saves cat owners from giving their cats a pill by offering a topical form of the drug that can be administered inside the animal's ear.

The pet medicine industry isn't just focused on small molecules. Aratana also has two USDA-approved monoclonal antibody treatments for B-cell and T-cell lymphomas in dogs, and KindredBio is developing biologics for cancer and allergies. In fact, biologics are so promising in people—accounting for 6 of the top 10 selling human drugs—that NexVet has decided to focus exclusively on developing them for pets. The company licenses human therapeutic antibodies and redesigns them for cats and dogs. To do so, NexVet researchers predict the minimal amino acid changes needed to adapt an antibody from one spe-

REGULATING ANIMAL THERAPEUTICS

As in humans, most pet medications are regulated by the US Food and Drug Administration (FDA). If the pharmaceutical product (usually an antibody or vaccine) acts through the immune system, the product will be regulated by the US Department of Agriculture (USDA). Both agencies seek to ensure that the product is both safe and effective in our animal companions, and continue to monitor the treatment once it's on the market. Here's what regulation involves at each agency:

FDA

- The FDA evaluates safety and effectiveness in a single, randomized, controlled trial (equivalent to a Phase 3 clinical trial in humans). Because the trials are small compared to those of human therapeutics (in a best-case scenario, a few hundred animals), the FDA evaluates all of the data collected during the trial.
- Companies can file for an extended-use protocol to allow pets who participated in the trial to continue the therapy after its completion and before regulatory approval.

USDA

- The USDA reviews all data provided by a company to determine safety and efficacy, inspects the manufacturing processes for the biologic, and performs its own tests of the products at USDA facilities.
- A product can be conditionally licensed, meaning that it is deemed safe with reasonable expectations of efficacy, or fully licensed if the efficacy requirement is also met. The conditional license provides companies with a year to demonstrate effectiveness and move towards full licensure; on occasion, the conditional license can be renewed.

cies to another to avoid immune rejection, and then engineer these proteins.

As more pet cancer therapies hit the market, animal-focused researchers are starting to think about how genomics might inform personalized pet medicine with newer diagnostic methods. For example, Massachusetts-based Innogenics provides a genomic test of canine cancer, ordered after standard histopathology, that probes the expression levels of nearly three dozen cancer-related genes. The test is designed to help guide decisions about what treatments might be effective, and to spur the development of new therapies based on the genetic patterns the test reveals. (A similar test for cats is in development.)

Another popular target in the field of pet medicine is pain. MediVet Biologics, which sells its own canine autologous cancer vaccine (K9-ACV), provides stem cell therapy kits for osteoarthritis in pets. Vets use the kit to turn adipose tissue they extract from pets into stem cells and inject them back into the animal. Data from a recent double-blind study suggest that the treatment—which, according to recent FDA draft guidance on autologous stem cell therapies, is self-regulated by the company—is effective. Vice president of business development Thomas Masterson says a single injection can manage symptoms for one to four years. In March 2016, Aratana received approval for its canine osteoarthritis small molecule drug Galliprant, and in August, the FDA cleared its canine injectable postoperative pain drug Nocita. And NexVet's most advanced products are anti-nerve growth factor (NGF) monoclonal antibodies that have shown safety and efficacy for treating chronic pain in a pivotal study of 246 dogs, and in a pilot study of 126 cats.

"Brand-new products are coming on the market for new indications," says the FDA's Troutman. "We are definitely seeing more applications" this year compared to last year, she adds.

. . . and back again

Human-focused pharmaceutical companies are increasingly embracing the idea that naturally occurring disease in pets could provide a better model for human disease than conditions introduced in the lab. "A lump of cancer cells under the skin of a mouse is not real cancer," says Kindred's Chin. By contrast, the cellular and genomic features of certain tumors (such as osteosarcoma and non-Hodgkin lymphoma) are so similar in people and pets—particularly dogs—that "on occasion, [they] are considered to be indistinguishable between species," notes the report of a 2015 workshop on comparative oncology hosted by the U.S. National Academy of Medicine's National Cancer Policy Forum (*Sci Trans Med*, 8:324ps5, 2016). (See "Pet Scans," *The Scientist*, April 2016.)

This realization has launched initiatives such as the National Cancer Institute's Comparative Oncology Program, which uses pet clinical trials of new cancer therapeutics to both treat pets and learn more about human cancer. And if these animals are going to be the subjects of clinical trials testing new cancer treatments, many researchers will argue, the animals should have access to those that work. "It does feel right that if a species [such as dogs or cats] has been exposed to the risks associated with testing a new agent, and it works in them, then the species should be the beneficiary of the drug," says University of California, Los Angeles's Barbara Natterson-Horowitz, who coauthored a 2013 book called *Zoobiquity* describing the overlap between human and animal medicine.

At the same time, pet therapeutics companies are considering forays back into the human medical space. VetDC owns the rights to develop one of its compounds (a PI3K and mTOR inhibitor being tested to treat a variety of cancers) for human use, for example, and NexVet has set up a side company, TevXen, to apply their antibody technology to human therapeutics.

"The field is still very young and right at the beginning of the growth phase," says Chin. "The trajectory is going to be pretty steep upwards." ■

Jenny Rood is a freelance science writer based in Cambridge, Massachusetts, and a development writer for the Broad Institute of MIT and Harvard.

Truth and Power

Are leading researchers driven more by the quest for knowledge or the pursuit of fame?

BY BRUNO LEMAITRE

It is rumored that Nobel Prize–winning molecular biologist Jacques Monod's final words were, "I seek to understand." The deathbed statement of this pioneering researcher seems to echo the idealistic scientific search for truth. But some of his close colleagues have suggested that seeking truth was only one part of Monod's story. Other contemporaries were struck by Monod's robust ego: his strong need to dominate others and his thirst for fame.

Even Monod's interpersonal relationships could be seen as optimal for a man in search of power. He married a well-connected woman—the sister-in-law of a famous geneticist. Later, he had an extramarital relationship with a skilled research assistant: an excellent way to keep tabs on his other colleagues in the institute. Monod also had an affair with a journalist at the French newspaper *Le Monde*, another advantageous relationship for someone desiring public recognition.

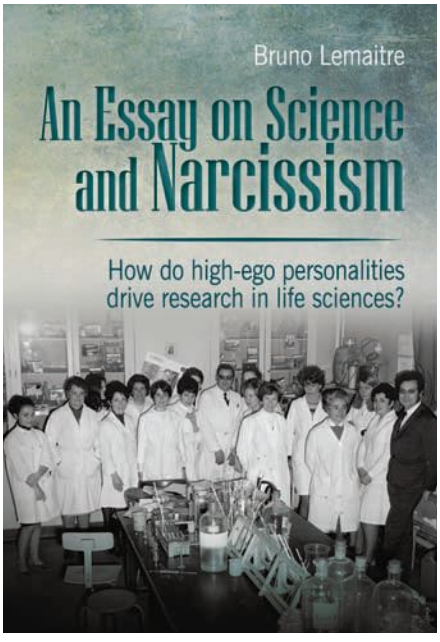
With his extreme self-confidence, domineering nature, and attention-seeking behavior, Monod might be described as a narcissist. In my latest book, *An Essay on Science and Narcissism*, I dissect how such traits influenced Monod and other scientific heavyweights.

Psychological studies show that narcissistic individuals tend to use human relationships to attain positions of authority or to improve their own visibility, as illustrated by Monod's strategic mate choice. The Monod case suggests that it would be naive to see scientists as simply seekers of truth. It is likely that the Nobel laureate enjoyed the position of power that science afforded him, and that it suited his personality. After all, a scientist is someone with expert knowledge who can reveal complex secrets to the public.

But how does narcissism, a personality trait associated with dominance and short-term mating strategies, influence the scientific process? As any minimally aware researcher will recognize, there are aspects of science, especially its politics, that are nonrational but that nevertheless have a tremendous impact on how science is actually practiced. This probably relates to the fact that some scientists are more inclined than others to battle for leadership positions. Scientists high in narcissism tend to use the scientific arena to reach positions of power for their own benefit rather than to serve the community.

But it would be erroneous to see this need for power in a purely negative light. The desire to get ahead probably contributed a great deal to Monod's scientific achievements. After all, the drive to succeed in science is likely a legacy of our evolutionary forebears, who were motivated to attain high social status for adaptive reasons. But the influences of narcissism on science could be more subtle, influencing career choice or strategies for achieving success. For example, scientists hungry for power are more likely to follow a clinical career path, to be attracted by jobs at elite institutions, or to gravitate to a fashionable field of research already in the spotlight. Scientists high on the narcissism scale are often better at networking, aggrandizing scientific notions or colleagues who are most likely to provide them with a direct benefit. Narcissistic researchers also tend to excel at using verbiage that resonates with the short-term expectations of politicians. Even as they pursue a career that prizes objectivity, narcissistic scientists may unconsciously distort reality to maintain a positive illusion about themselves. The growing trend of irreproducibility and overexaggeration in science could be explained by the need of narcissistic personalities to reach the status they believe they deserve.

These are only some of the fascinating questions related to the influence of personality on the conduct of science. The scientific enterprise is an ideal environment for observing such influences in the context of the objective quest for knowledge. ■



Bruno Lemaitre, April 2016

COURTESY OF BRUNO LEMAITRE

Bruno Lemaitre studies insect immunity at the Ecole Polytechnique Fédérale of Lausanne in Switzerland, where he's also developed a strong interest in psychology and epistemology. Read an excerpt of An Essay on Science and Narcissism: How Do High-Ego Personalities Drive Research in Life Sciences? *at the-scientist.com.*

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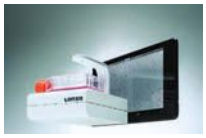

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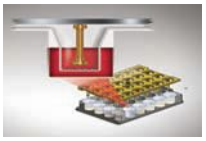

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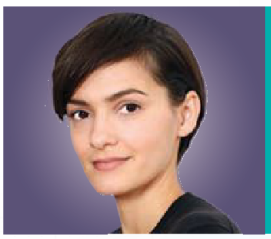

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First Transgenic *Arabidopsis*, 1986

BY KERRY GRENS

In the fall of 1982, Robert Horsch, a tissue-culture expert hired by Monsanto a year earlier, came bursting out of the lab's plant-growing room. Clutching a handful of experimental specimens, Horsch ran up and down the hallway yelling, "It worked! It worked! It worked!" Robb Fraley, Horsch's colleague who is now Monsanto's chief technology officer, recalls that special day. "I don't think anyone was confused about what was happening. It was magical."

What had worked was a transformation protocol that Fraley, Horsch, and their colleagues had developed to insert a foreign gene into petunia and tobacco plants using *Agrobacterium tumefaciens* (*PNAS*, 80:4803-07, 1983). Their success in developing what is still a go-to method for genetic engineering marked the beginning of a new era in agriculture and basic plant biology.

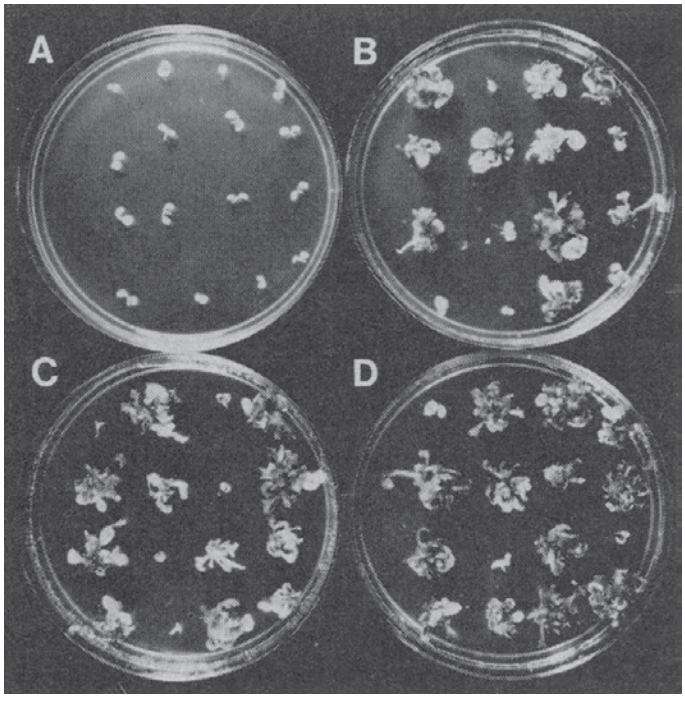
At the time, Monsanto was not known for any molecular-biology prowess, but rather for its agricultural chemical manufacturing. In 1981, the firm put together a small biotechnology dream team tasked with figuring out how to put genes into plants, with Fraley as the DNA-delivery expert, Horsch leading the way in tissue culture, and Steve Rogers pioneering recombinant DNA.

"What made it challenging and exciting and a scientific first was, if you think about it, we not only had to develop the method for getting a gene into a plant cell, but the ability to detect that gene and determine if it had been passed on into the tissues of the plant that allowed it to regenerate and create new plants and pass those seeds on to the next generation," says Fraley. "For it to work, we had to solve all those problems and solve them simultaneously."

Fraley's group was among several that had been working toward genetically engineering plants. A banner year for the field, 1983 saw Mary-Dell Chilton's team at Washington University in St. Louis, Marc van Montagu's group at Ghent University in Belgium, and Fraley's Monsanto crew each publish significant advances in quick succession. (These three scientists ended up sharing the 2013 World Food Prize for their accomplishments.)

The next step for the Monsanto group was to move beyond the Solanaceae family of plants. Horsch, now head of the agricultural research and development team at the Gates Foundation, recalls that *Arabidopsis thaliana* was looking like it would become a facile model organism for plant science. So when Alan Lloyd, a greenhouse manager at Monsanto, came to Horsch asking for a project, he suggested that Lloyd work on applying the transformation technique to the little plant.

"If a greenhouse manager could figure out how to do this, I figured that was a really good indication that a lot of people could do this," says Horsch. By testing various tissue culture conditions, selectable markers, and *Agrobacterium* strains, Lloyd succeeded. In 1986, the team published the protocol (*Science*, 234:464-66), along with the bold and prescient statement that "demonstrations of the feasibility of these approaches to the identification, isolation, and analysis of specific plant genes will guarantee the position of *A. thaliana* as 'the *Escherichia coli* of the plant kingdom.'" Indeed. ■



TRANSFORMING PLANT BIOLOGY: In 1986, three years after producing transgenic petunia and tobacco plants using *Agrobacterium*, a team from Monsanto developed a similar protocol to transform *Arabidopsis*. The researchers inserted a gene into the plant that would confer resistance to the antibiotic hygromycin. They then grew plants on medium that selects for hygromycin resistance. Wild-type plants (A) grew poorly, while most of the progeny of transgenic plants thrived (B-D).

Bottom, from left to right: Monsanto collaborators Rogers, Roger Beachy, Horsch, and Fraley, at the first biotech crop test plot, 1987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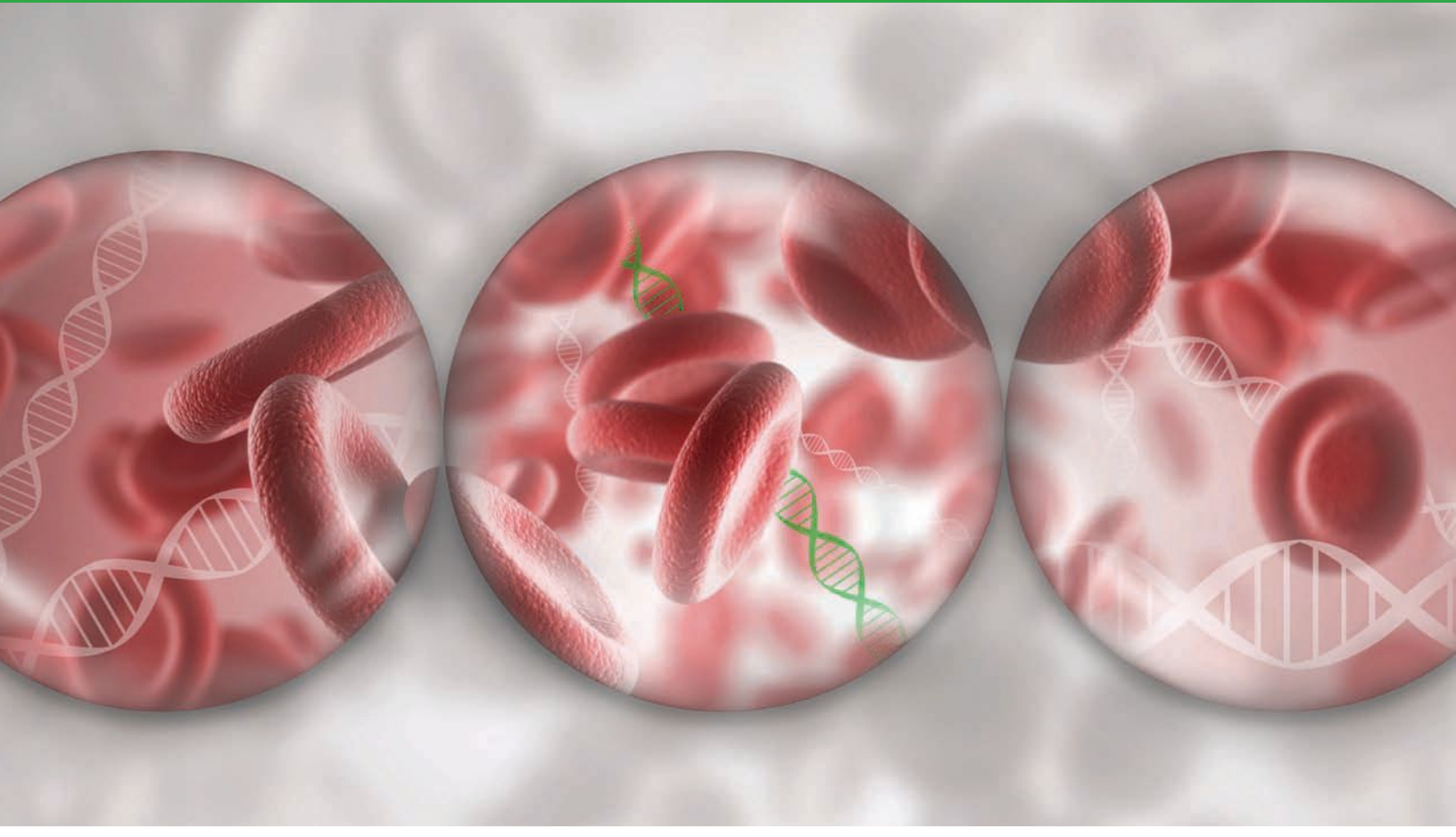

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