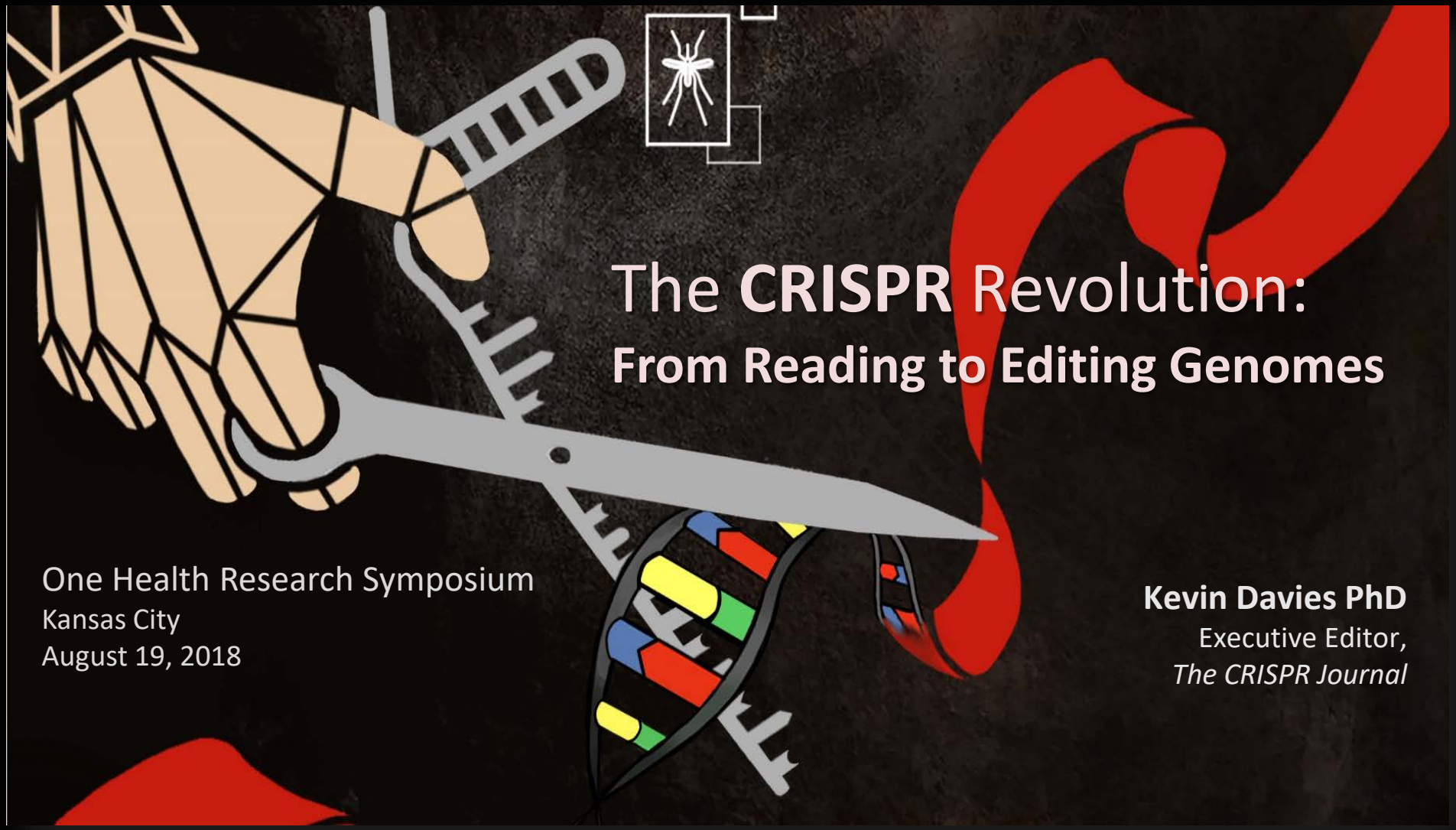


The **CRISPR** Revolution:
From Reading to Editing Genomes

One Health Research Symposium
Kansas City
August 19, 2018

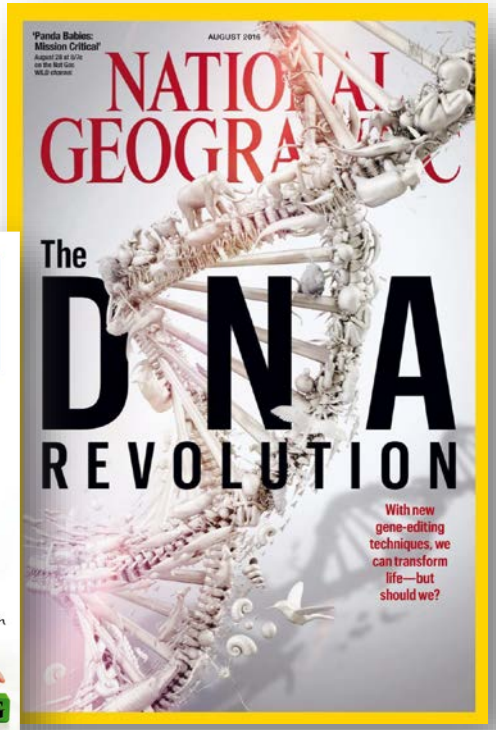
Kevin Davies PhD
Executive Editor,
The CRISPR Journal


Cell
Volume 167
Number 7
December 15, 2016
www.cell.com
ANTI-CRISPR
nature
APRIL 2014
CRISPR TECHNOLOGY
REVOLUTIONARY GENE MODIFICATION
Fighting hereditary diseases at the source and cleaving DNA with surgical precision PAGE 11
BURN BOOK: SECRETS REVEALED
Streptococcus & Escherichia coli bacterial genomes provide insight into genome editing
ON WEDNESDAYS WE WEAR PINK
Commercialization of the Pink Ribbon?
New hope for China's left-behind kids p. 1226
How pesticides should be regulated p. 1232
A twist on photoemission delay pp. 1239 & 1274
Science
$15
22 SEPTEMBER 2017
sciencemag.org
AAAS
CRISPR PIGS
Eliminating endogenous retrovirus in a step toward xenotransplantation
pp. 1238 & 1303

CRISPR


TIME
The Gene Machine
What the CRISPR experiments mean for humanity

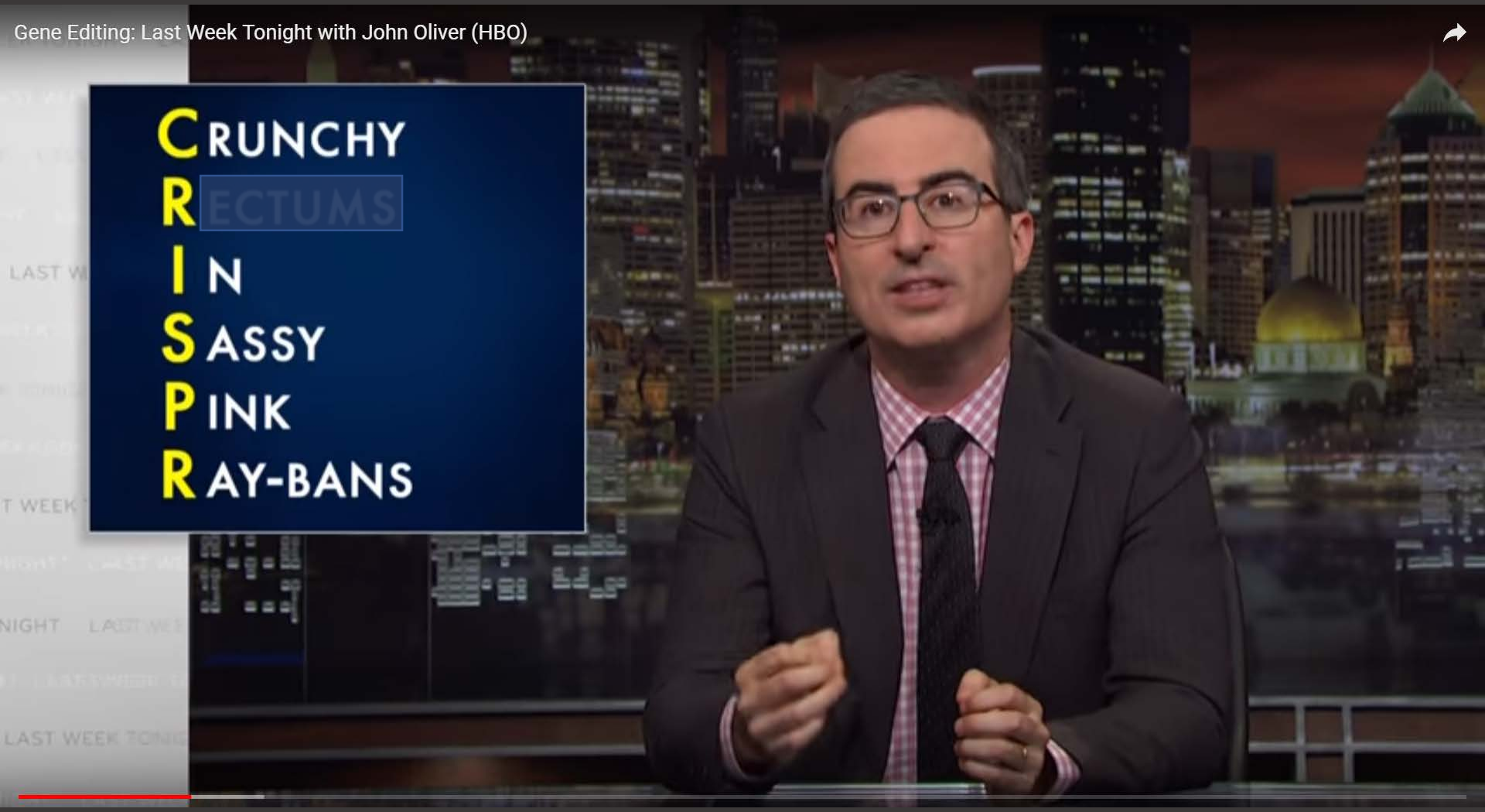

The Economist
How Russians cope with recession
No-go for NGOs in China
Islamic State's taste for slavery
Commodities: the binge, the hangover
India's poet-politicians
Editing humanity
The prospect of genetic enhancement
High IQ
No baldness
Low risk of Alzheimer's, breast cancer and strokes
Perfect pitch
Sprinter
20/20 vision


'Panda Babies: Mission Critical'
AUGUST 2015
NATIONAL GEOGRAPHIC
The DNA REVOLUTION
With new gene-editing techniques, we can transform life—but should we?


No hunger.
No pollution.
No disease.
WIRED
And the end of life as we know it.
The Genesis Engine.


C&EN
CHEMICAL & ENGINEERING NEWS
DISRUPTING PRIONS
Small molecules target progressive diseases P.37
BOSTON IN PHOTOS
Sights from the fall 2015 ACS national meeting P.48
EDITING THE GENOME
CRISPR/Cas9 takes gene manipulation into a new era P.14


The risks of using CRISPR to edit embryos
ENERGYGUIDE
60 MINUTES Overtime
Exit full screen


Gene Editing: Last Week Tonight with John Oliver (HBO)
CRUNCHY
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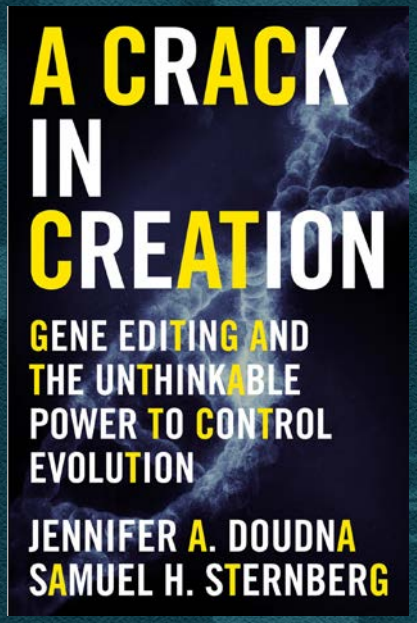
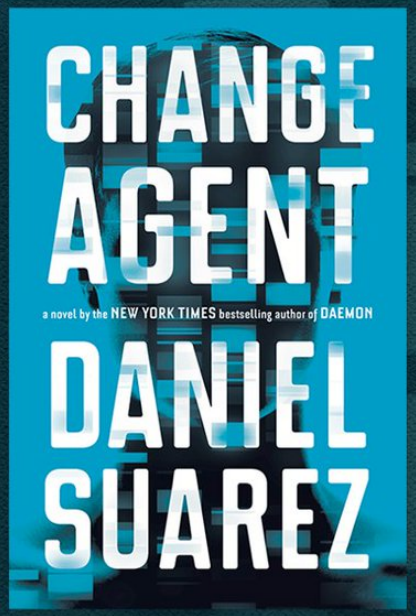
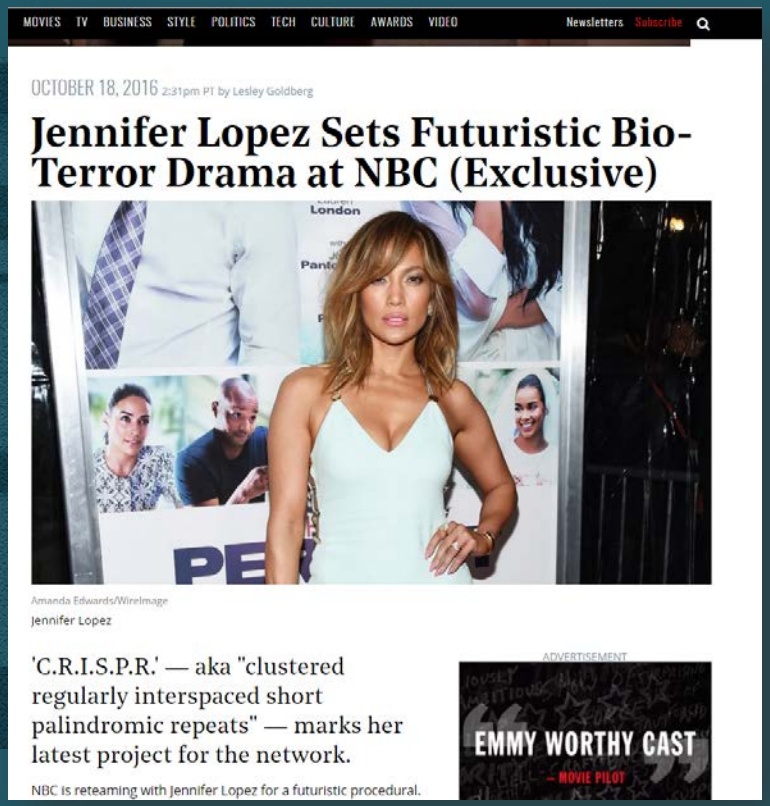
SASSY
PINK
RAY-BANS

CRISPR in the Public Eye







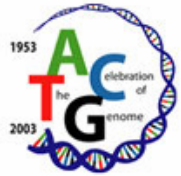
"Are you familiar with CRISPR?"

We've gone from reading DNA…

genome.gov
National Human Genome Research Institute

April 14, 2003

Newsroom



National Human Genome Research Institute
National Instututes of Health
Department of Health and Human Services
and
Office of Science
U.S. Department of Energy

International Consortium Completes Human Genome Project

All Goals Achieved; New Vision for Genome Research Unveiled

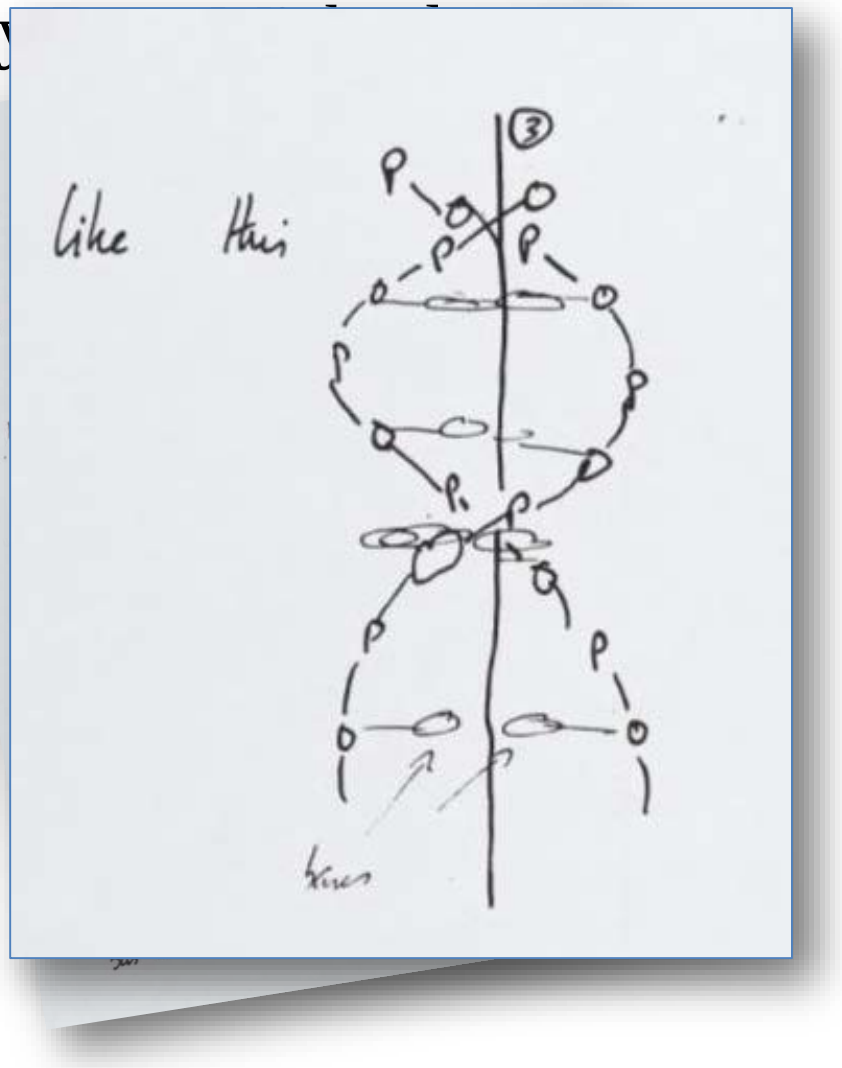
BETHESDA, Md., April 14, 2003 - The International Human Genome Sequencing Consortium, led in the United States by the National Human Genome Research Institute (NHGRI) and the Department of Energy (DOE), today announced the successful completion of the Human Genome Project more than two years ahead of schedule.

Also today, NHGRI unveiled its bold new vision for the future of genome research, officially ushering in the era of the genome. The vision will be published in the April 24 issue of the journal *Nature*, coinciding with the 50th anniversary of *Nature's* publication of the landmark paper by Nobel Laureates James Watson and Francis Crick that described DNA's double helix. Dr. Watson also was the first leader of the Human Genome Project

The international effort to sequence the 3 billion DNA letters in the human genome is considered by many to be one of the most ambitious scientific undertakings of all time, even compared to splitting the atom or going to the moon.

"The Human Genome Project has been an amazing adventure into ourselves, to understand our own DNA instruction book, the shared inheritance of all humankind," said NHGRI Director Francis S. Collins, M.D., Ph.D., leader of the Human Genome Project since 1993. "All of the project's goals have been completed successfully - well in advance of the original deadline and for a cost substantially less than the original estimates."

“My



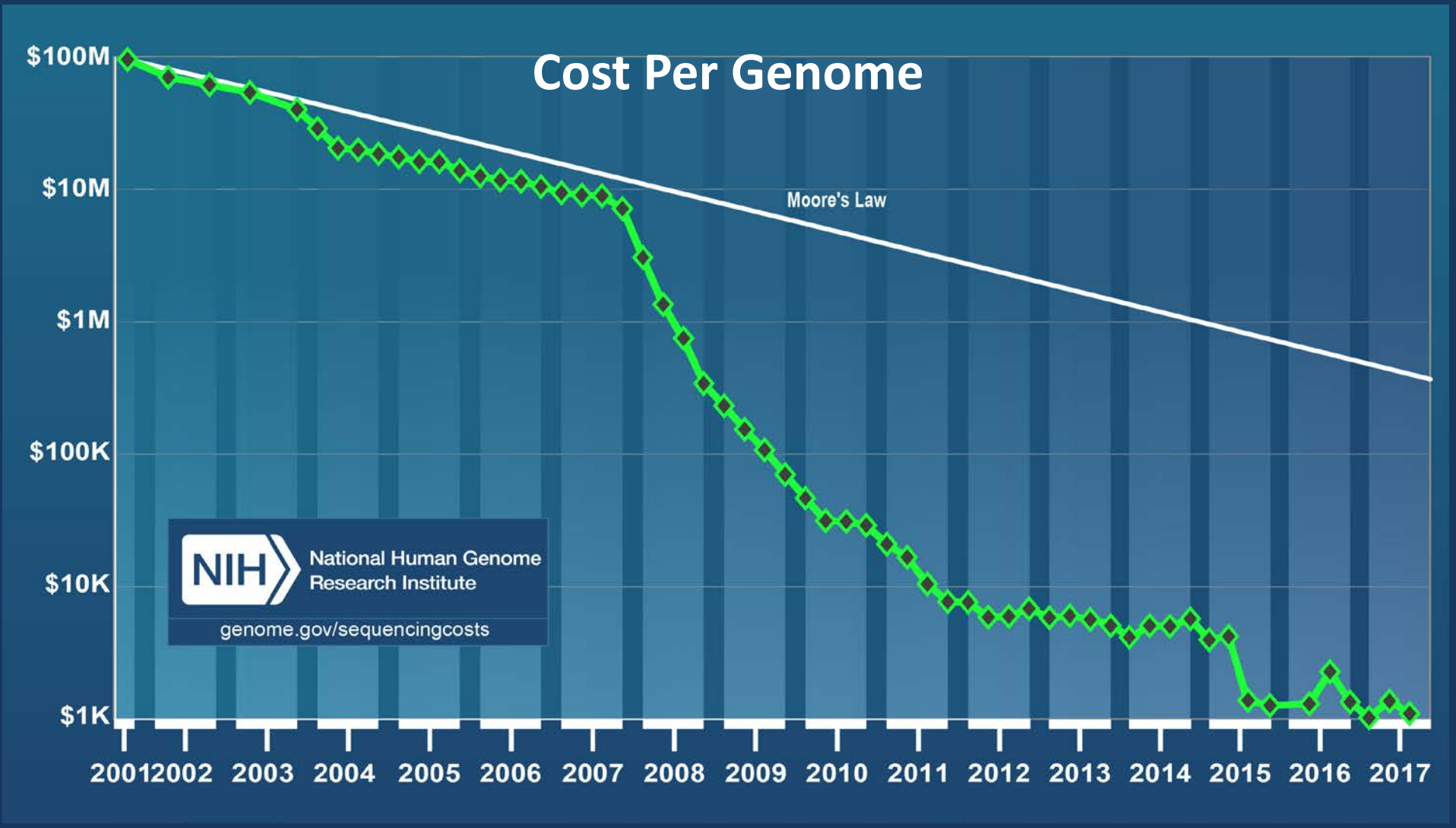
19 March 1953

Jim Watson and I have probably made a most important discovery…

Our structure is very beautiful. D.N.A. can be thought of roughly as a very long chain with flat bits sticking out. The flat bits are called the ‘bases’…

… Lots of love, Daddy”

Cas Kramer (Univ Leicester)


Cost Per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001
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“The Chemists’ Pub”

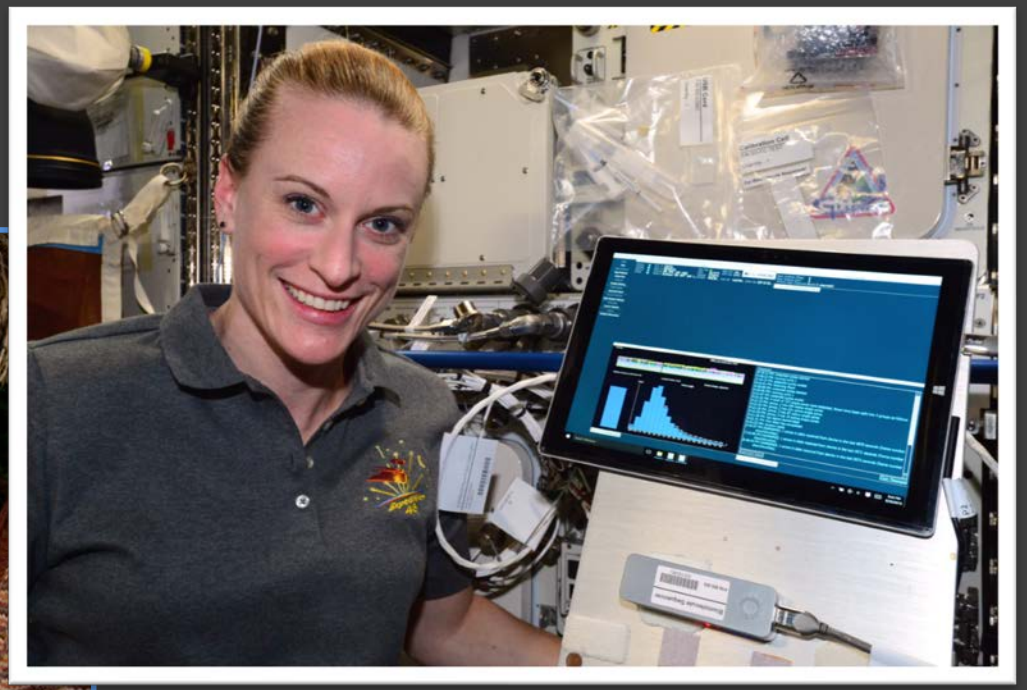
Sir Shankar Balasubramanian & David Klenerman

The Panton Arms | University of Cambridge, UK

“Project Jim”

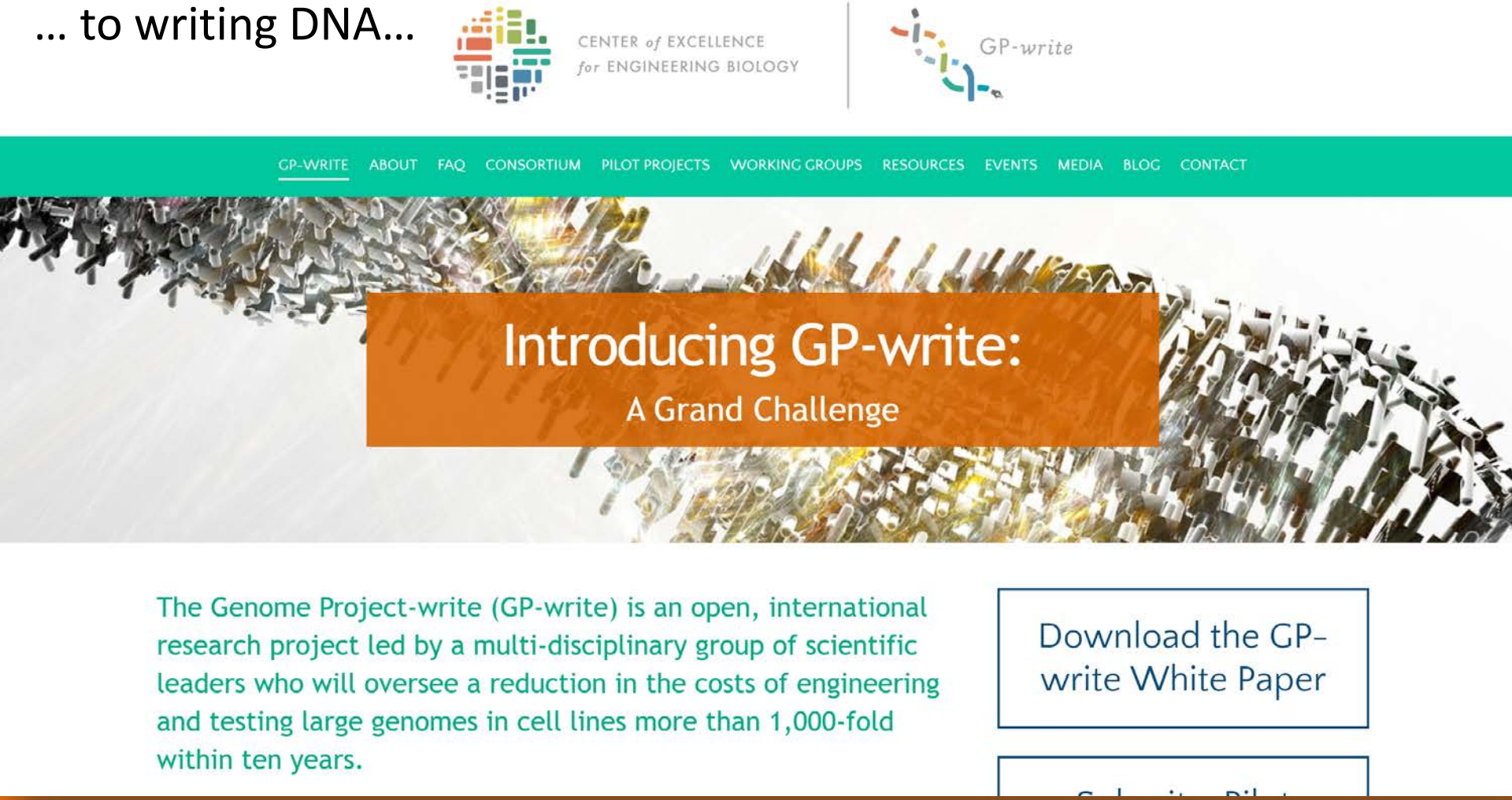
Houston
31 May 2007

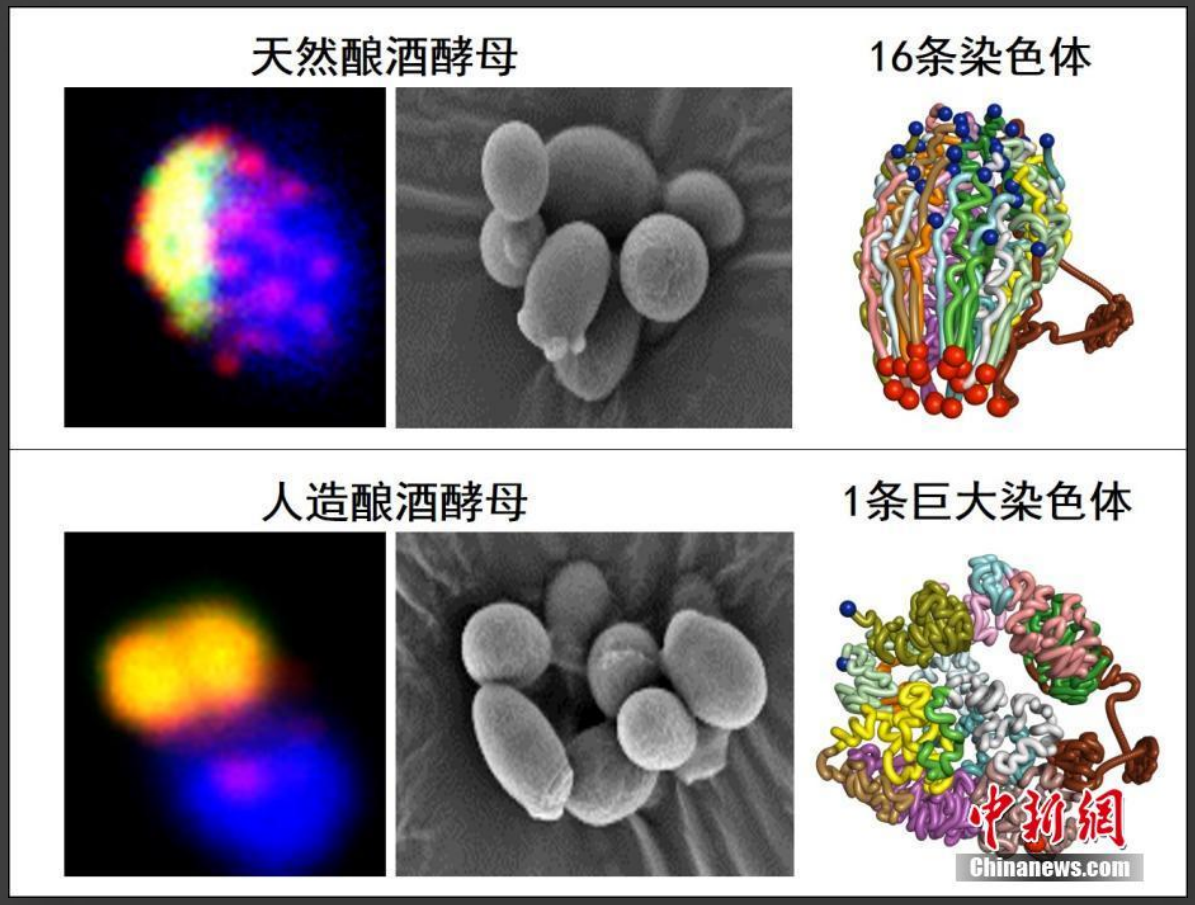
The Portable Sequencer



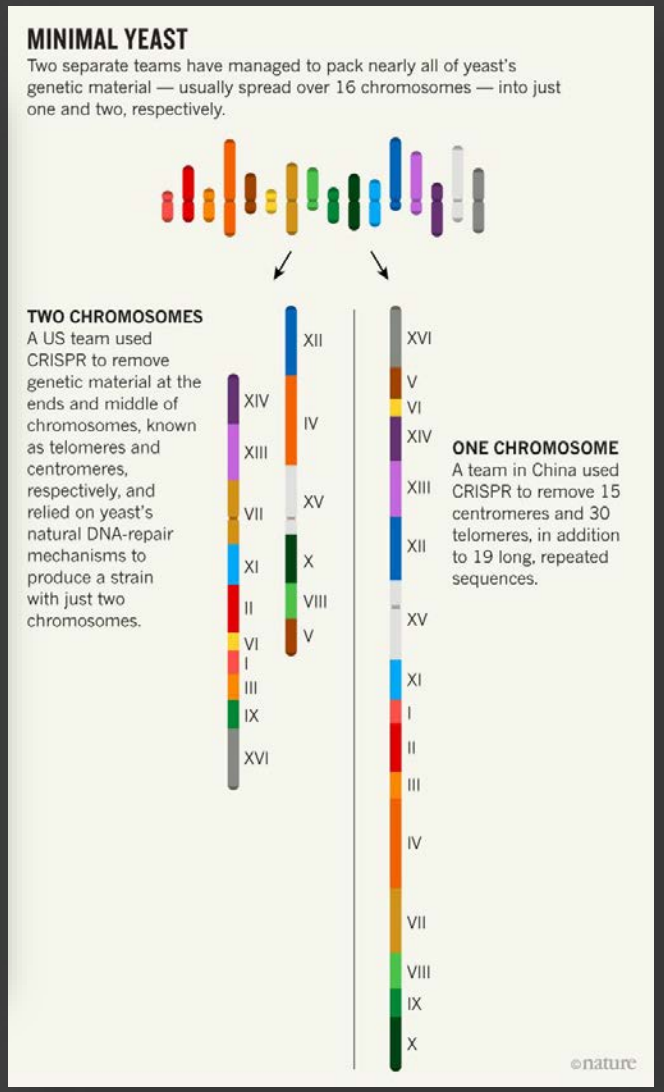
Quick et al. *Nature* February 2016

… to writing DNA…

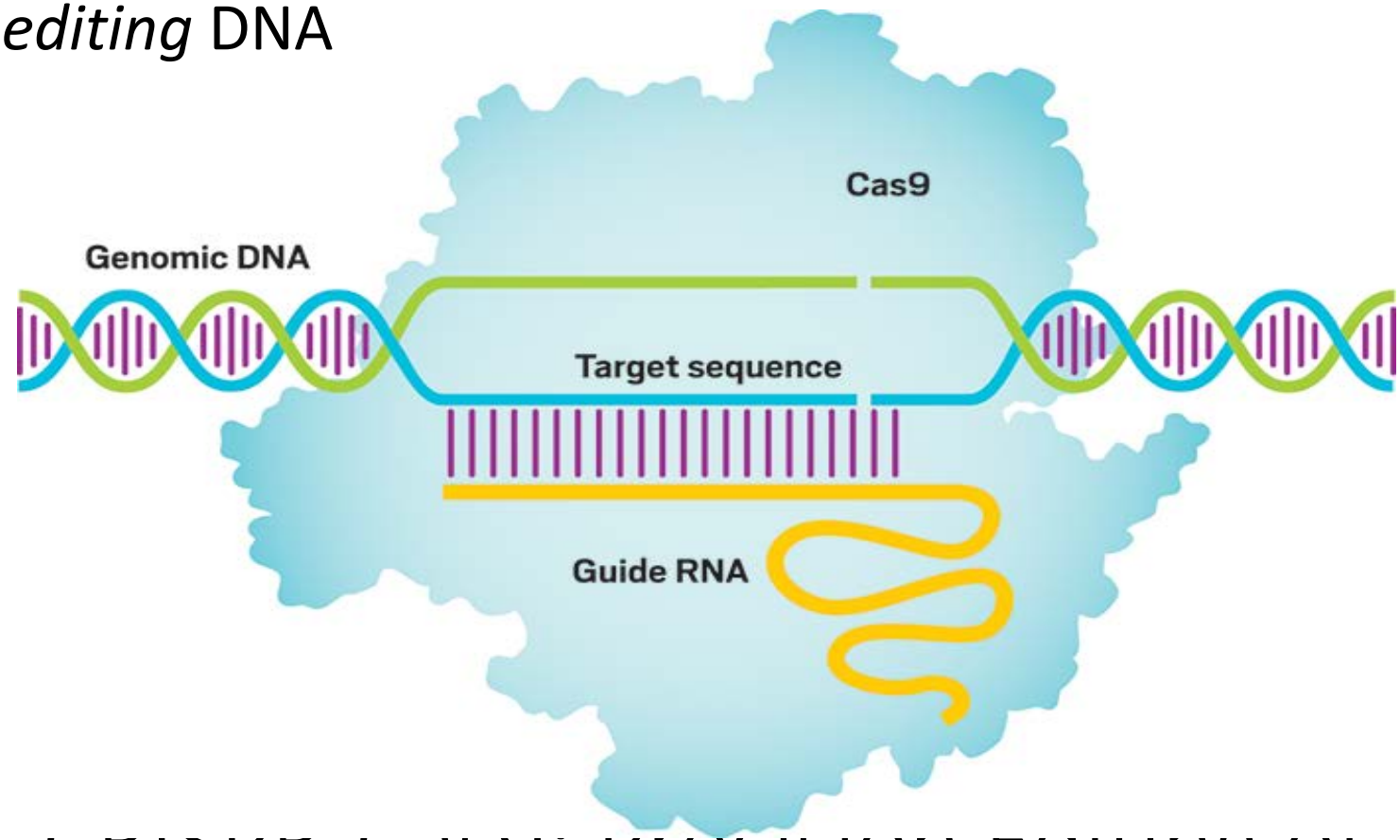




Shao *et al.*, Luo *et al. Nature* July 2018

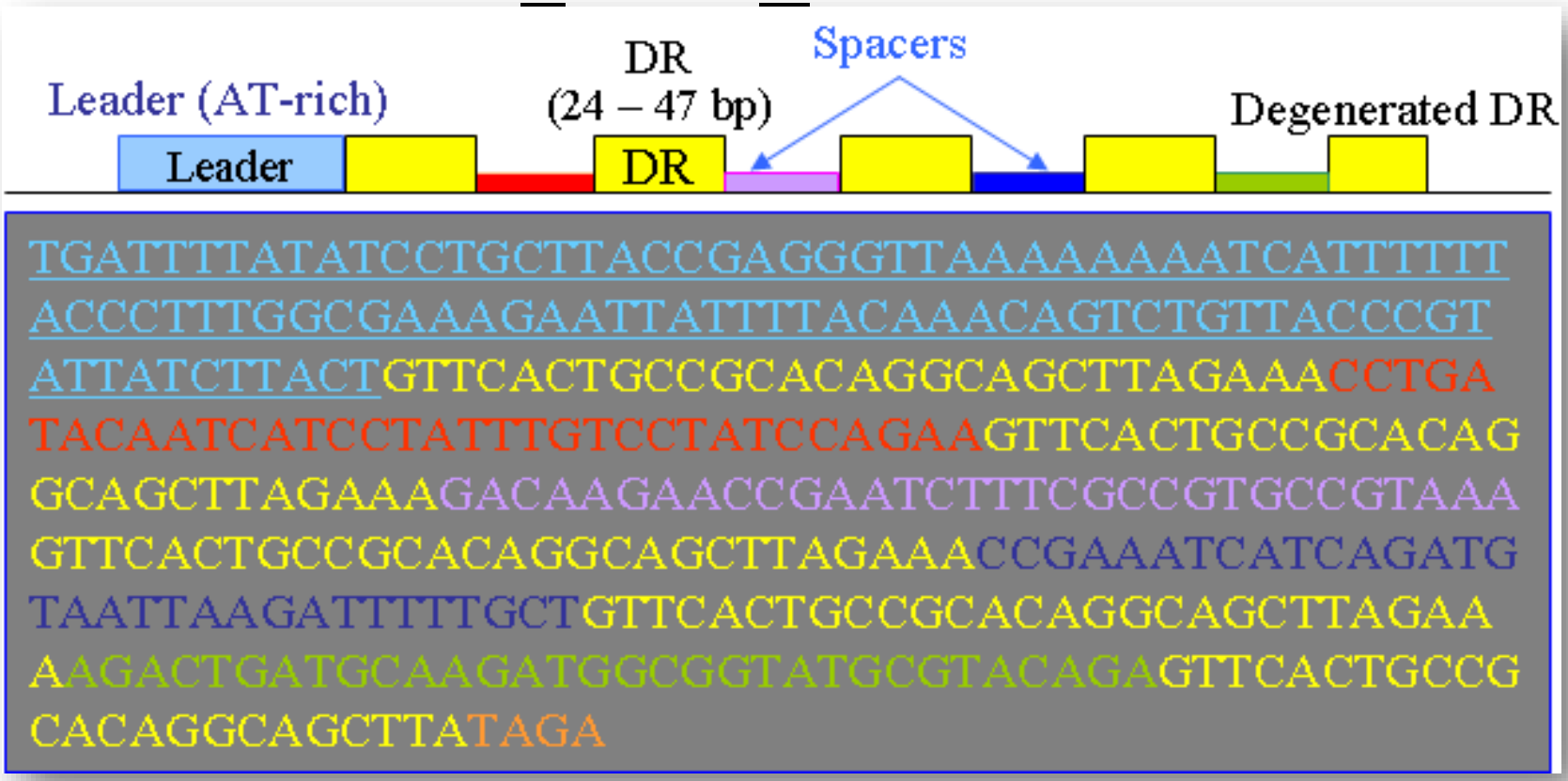


… to *editing* DNA



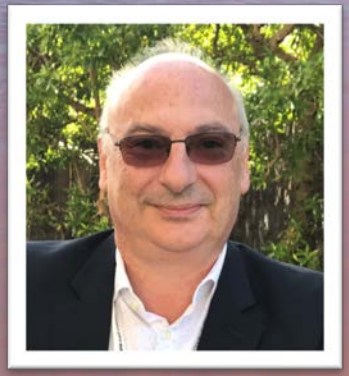
CRISPR-Cas9: Precision Editing of DNA

CRISPR: Clustered Regularly Interspaced Short Palindromic



Francisco Mojica

Salt Lakes of Santa Pola



Asunto: **Re: Acronym**
Fecha: Wed, 21 Nov 2001 16:39:06 +0100
De: "Ruud Jansen" <R.Jansen@vet.uu.nl>
Empresa: Diergeneeskunde
A: "Francisco J. Martínez Mojica" <fmojica@ua.es>

Dear Francis

What a great acronym is CRISPR.
I feel that every letter that was removed in the alternatives made it less crispy so I prefer the snappy CRISPR over SRSR and SPIDR.
Also not unimportant is the fact that in MedLine CRISPR is a unique entry, which is not true for some of the other shorter acronyms.

JOURNAL OF MOLECULAR EVOLUTION

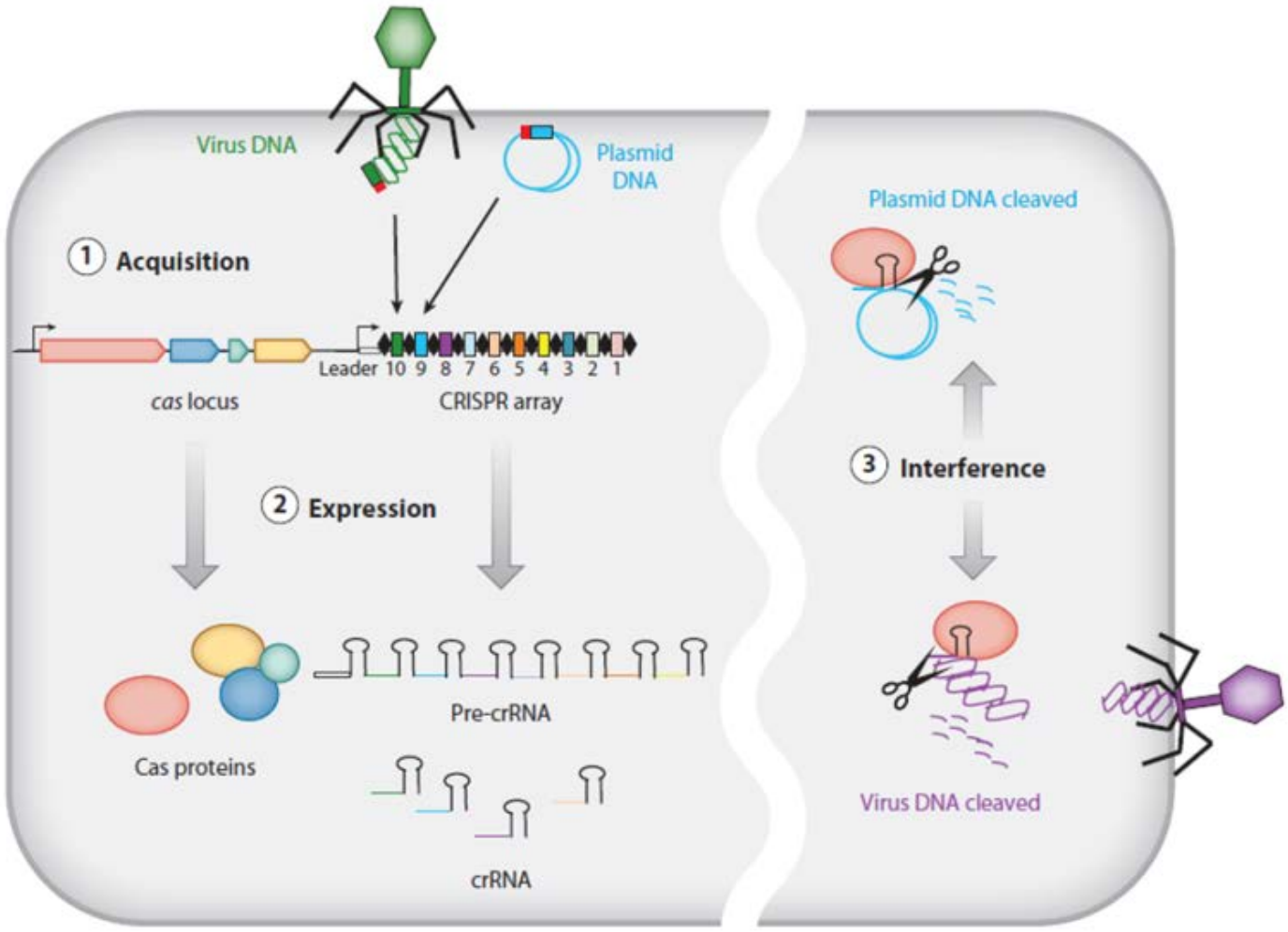
Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements

Francisco J.M. Mojica, César Díez-Villaseñor, Jesús García-Martínez, Elena Soria

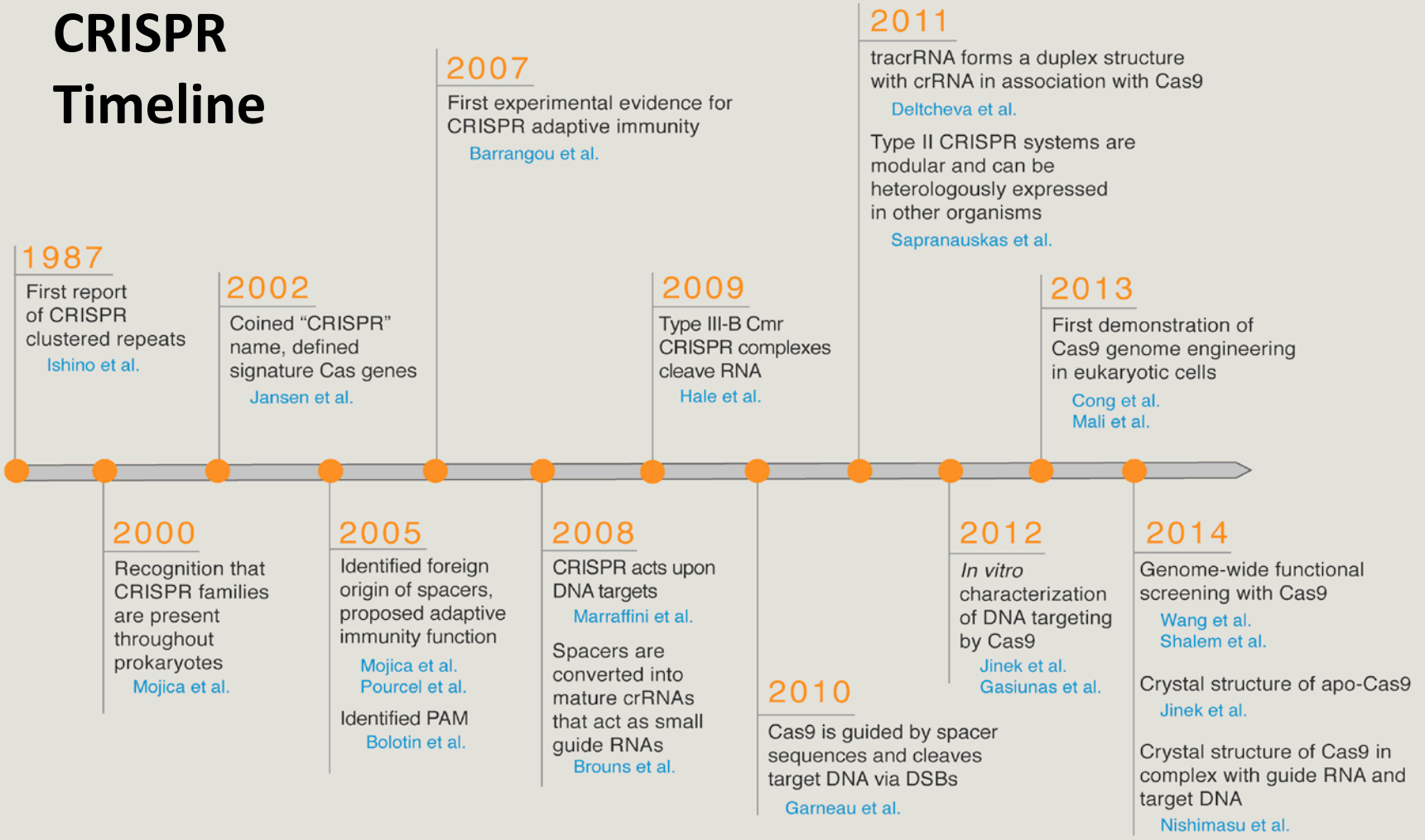
División de Microbiología, Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Campus de San Vicente, E-03080, Spain

Received: 6 February 2004 / Accepted: 1 October 2004 [*Reviewing Editor:* Dr. John Huelsenbeck]

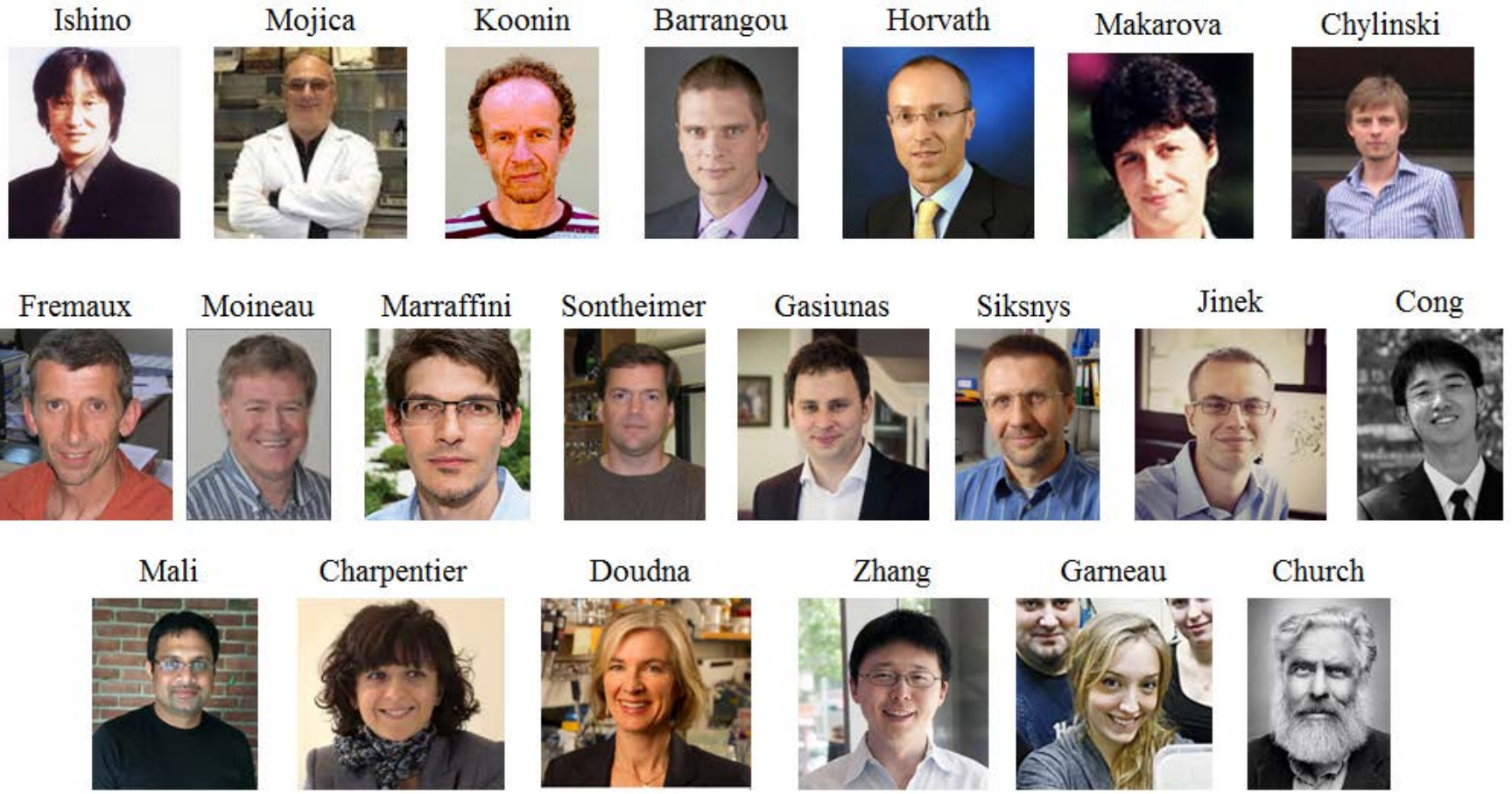
CRISPR is a naturally occurring, bacterial immune defense system that provides a means to recognize, remember and destroy viral invaders



CRISPR Timeline



Heroes of CRISPR

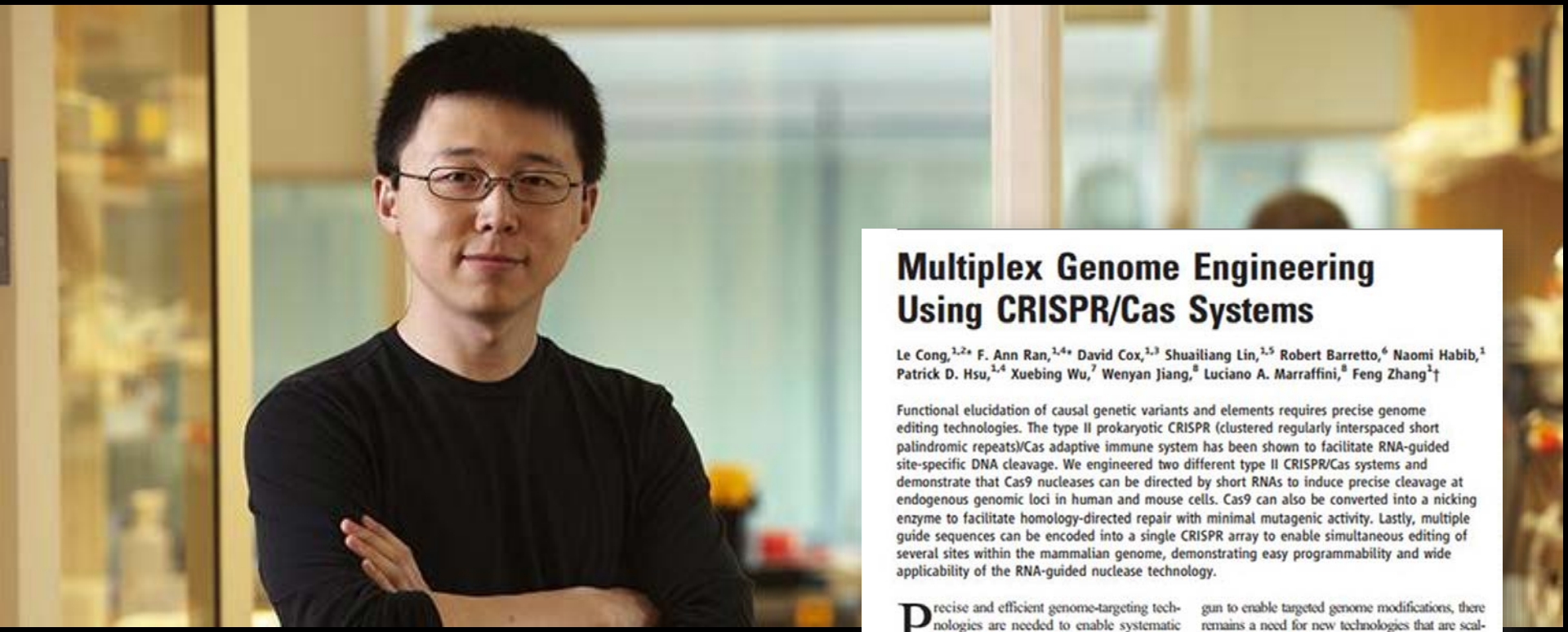


Emmanuelle Charpentier Jennifer Doudna

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2*] Krzysztof Chylinski,[3,4*] Ines Fonfara,[4] Michael Hauer,[2†] Jennifer A. Doudna,[1,2,5,6‡] Emmanuelle Charpentier[4‡]

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) syst provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in



Feng Zhang (MIT/Broad Institute)

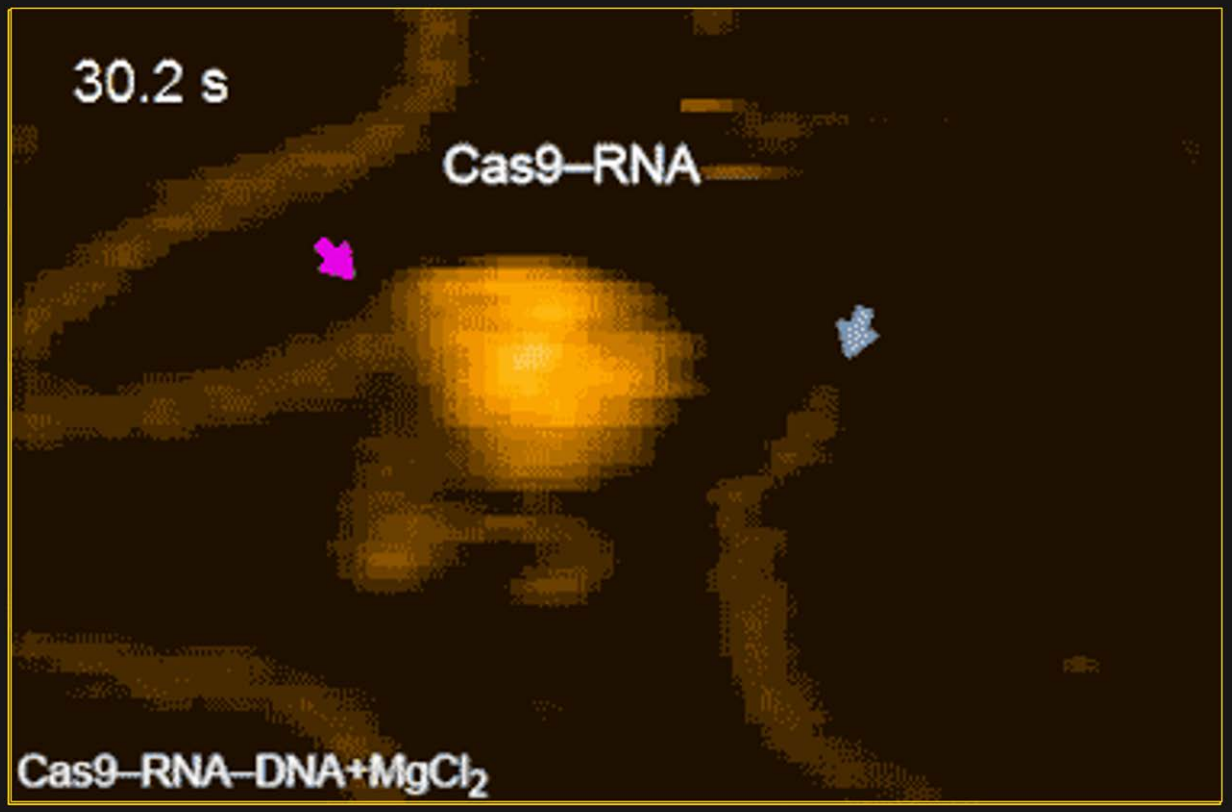
Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2*] F. Ann Ran,[1,4*] David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

Functional elucidation of causal genetic variants and elements requires precise genome editing technologies. The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)/Cas adaptive immune system has been shown to facilitate RNA-guided site-specific DNA cleavage. We engineered two different type II CRISPR/Cas systems and demonstrate that Cas9 nucleases can be directed by short RNAs to induce precise cleavage at endogenous genomic loci in human and mouse cells. Cas9 can also be converted into a nicking enzyme to facilitate homology-directed repair with minimal mutagenic activity. Lastly, multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology.

Precise and efficient genome-targeting technologies are needed to enable systematic reverse engineering of causal genetic variations by allowing selective perturbation of individual genetic elements. Although genome-editing technologies such as designer zinc fingers (ZFs) (*1*–*4*), transcription activator–like effectors (TALEs) (*4*–*10*), and homing meganucleases (*11*) have begun to enable targeted genome modifications, there remains a need for new technologies that are scalable, affordable, and easy to engineer. Here, we report the development of a class of precision genome-engineering tools based on the RNA-guided Cas9 nuclease (*12*–*14*) from the type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system (*15*–*18*).

Lights. Camera. Action… **CUT!**



CRISPR-Cas9 visualized by high-speed atomic force microscopy

M. Shibata, H. Nishmasu *et al*.
Nature Communications 8, 1430 (2017)

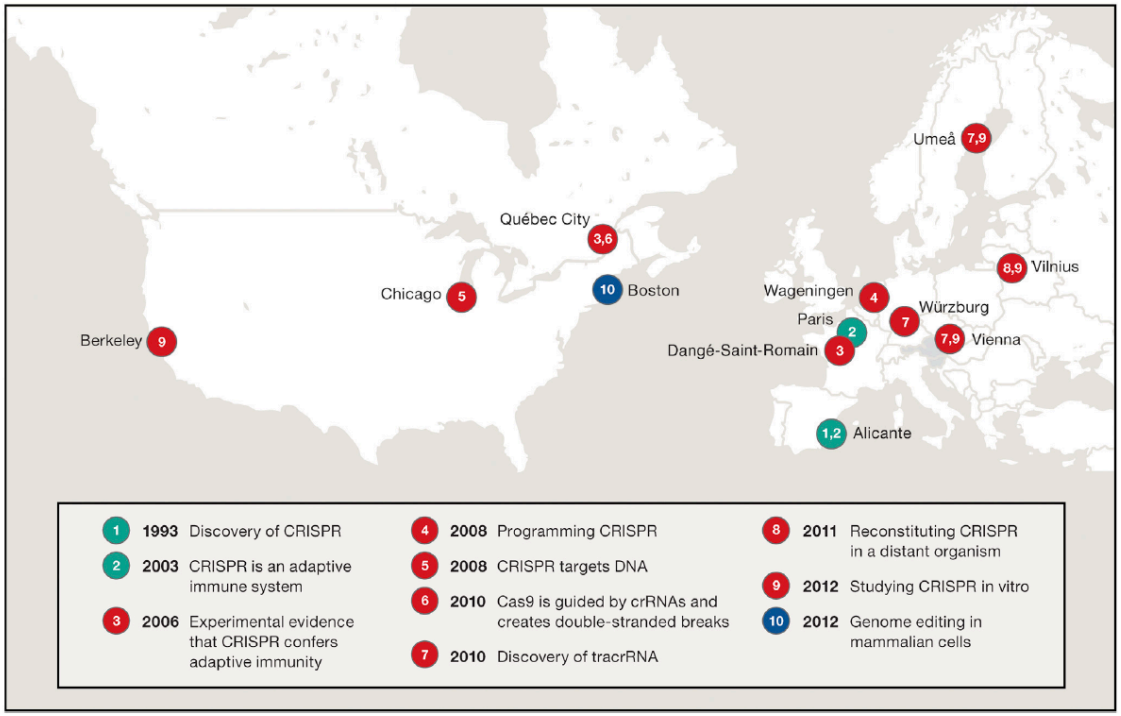
Hiroshi Nishimasu (Univ Tokyo)

Cell

The Heroes of CRISPR

Eric S. Lander[1,2,3,*]
[1]Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, M
[2]Department of Biology, Massachusetts Institute of Technology, Ca
[3]Department of Systems Biology, Harvard Medical School, Boston,
*Correspondence: lander@broadinstitute.org
http://dx.doi.org/10.1016/j.cell.2015.12.041

Three years ago, scientists reported that CRIS
genome editing in living eukaryotic cells. Since th
by storm, with thousands of labs using it for app
preceding 20-year journey—the discovery of a s
as an adaptive immune system; its biological ch
gineering—remains little known. This Perspectiv
and the stories of pioneers—and draw lessons a
tific discovery.



Lander's essay "is his masterwork, at once so evil and yet so brilliant that I find it hard not to stand in awe even as I picture him cackling loudly in his Kendall Square lair, giant laser weapon behind him poised to destroy Berkeley if we don't hand over our patents." --- **Michael Eisen** (HHMI/UC Berkeley)

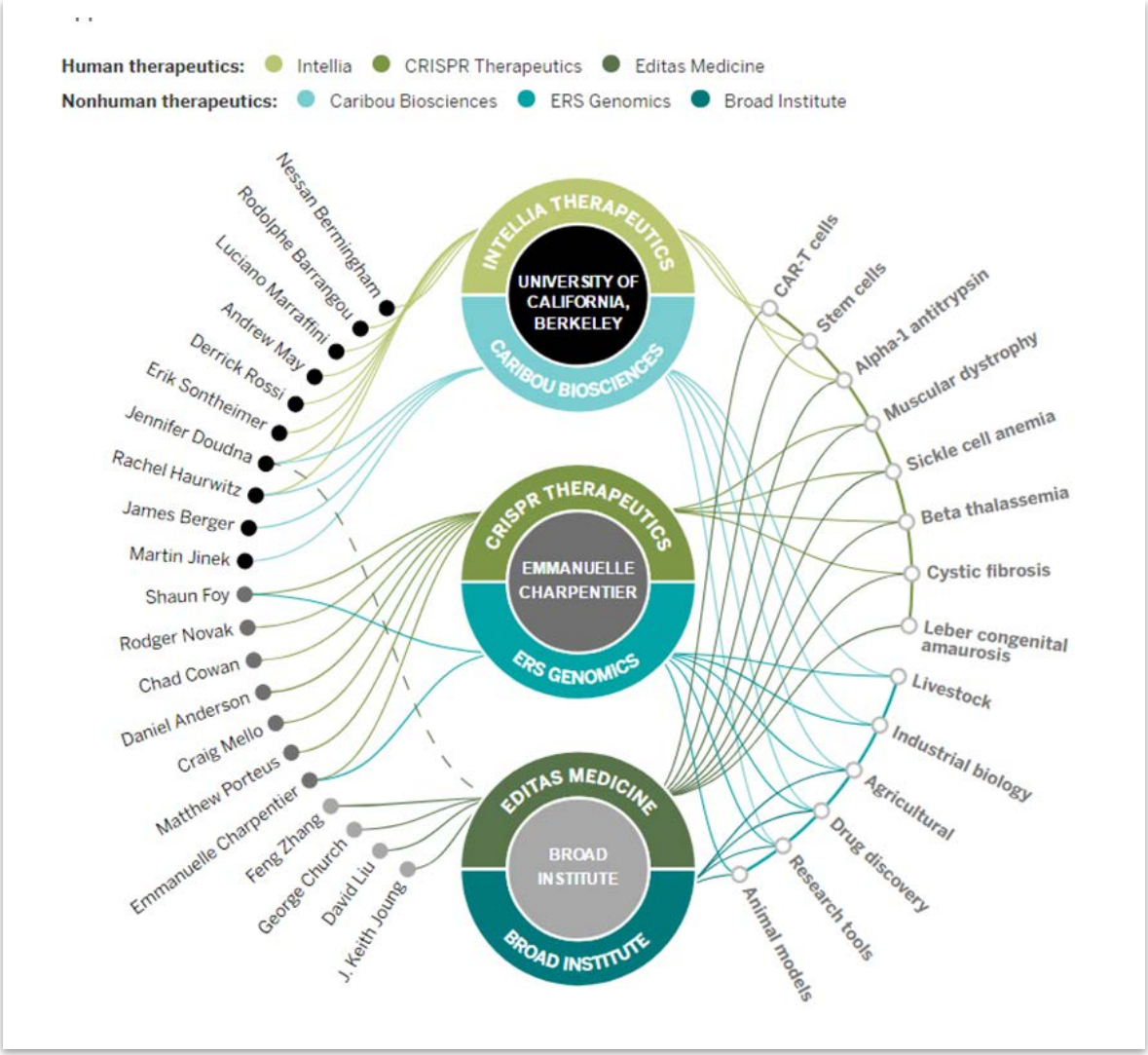
CRISPR Business

CARIBOU BIOSCIENCES®

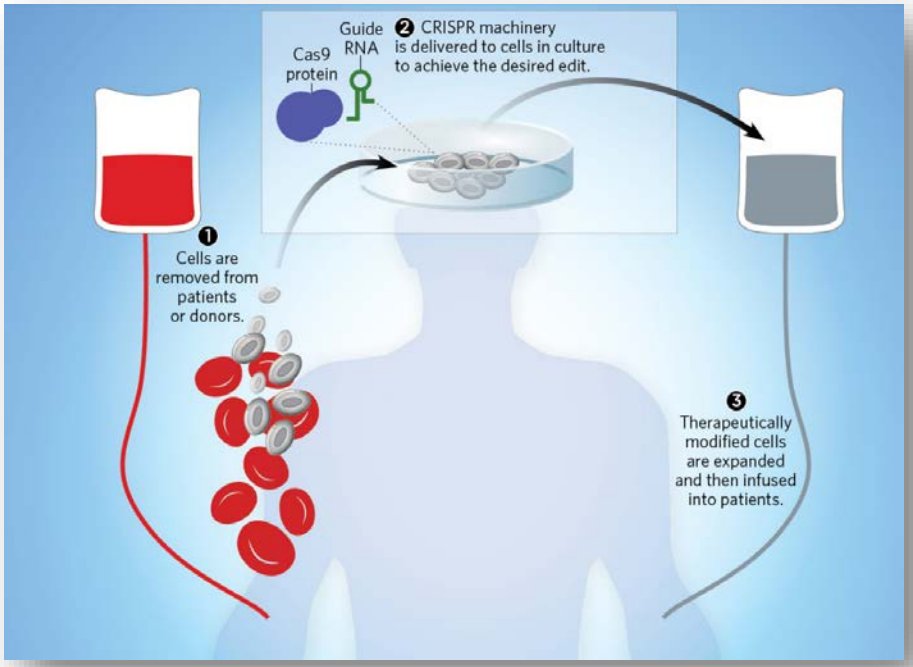
Intellia THERAPEUTICS (Doudna)

CRISPR THERAPEUTICS (Charpentier)

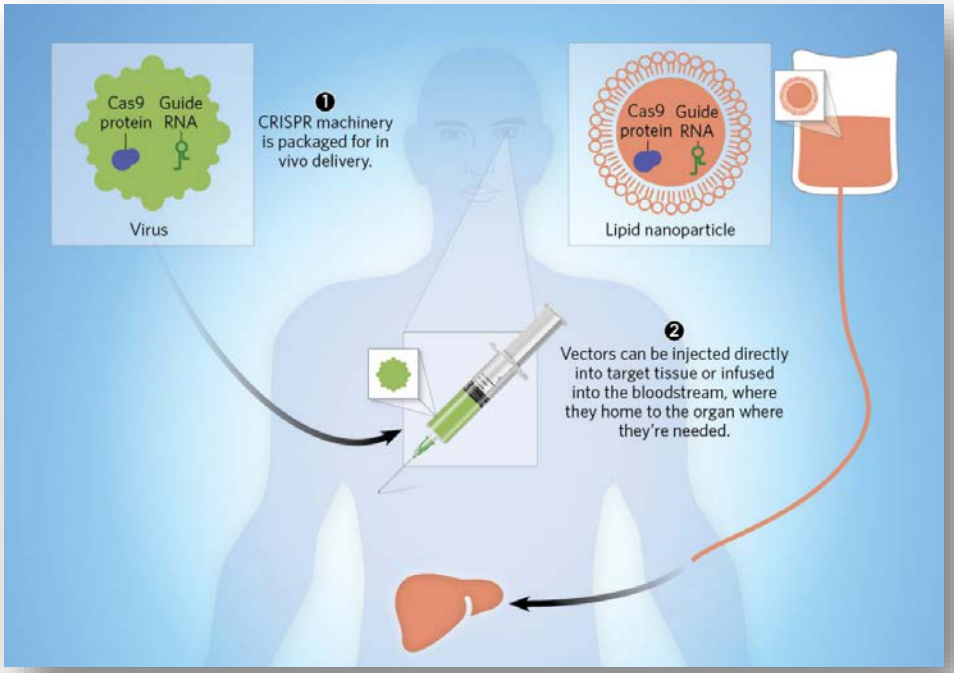
editas MEDICINE (Zhang)



CRISPR Gene Therapy



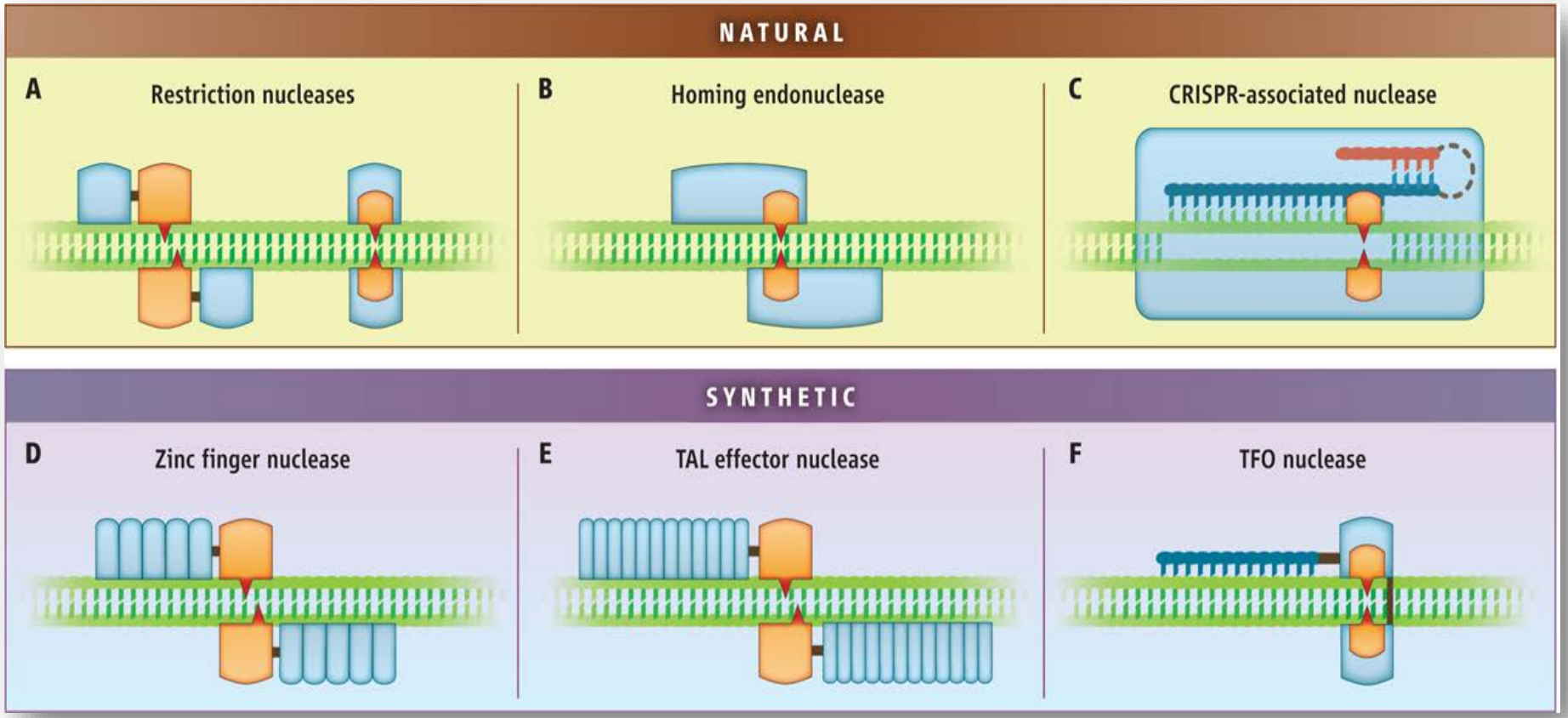
ex vivo



in situ

The Scientist

Genome Editing B.C. (Before CRISPR)



van der Oost J. *Science* Feb 2013

“Invisible Mending”

Home » Latest News » National News » AP Exclusive: US scientists...

AP Exclusive: US scientists try 1st gene editing in the body

AP By The Associated Press
November 15, 2017 7:48 am

Brian Madeux, 44, uses an infrared device to look at his veins as nurse Siobhan Field prepares an IV line for the first human gene editing therapy for NPS, at the UCSF Benioff Children's Hospital Oakland in Oakland, Calif., on Monday, Nov. 6, 2017. Madeux, who...

OAKLAND, Calif. (AP) — Scientists for the first time have tried editing a gene inside the body in a bold attempt to permanently change a person's DNA to try to cure a disease.

“It’s kind of humbling. I’m willing to take that risk. Hopefully it will help me and other people.”

--- Brian Madeux, 44

Hunter Syndrome patient

Associated Press, Nov 15, 2017

Luhan Yang (eGenesis)

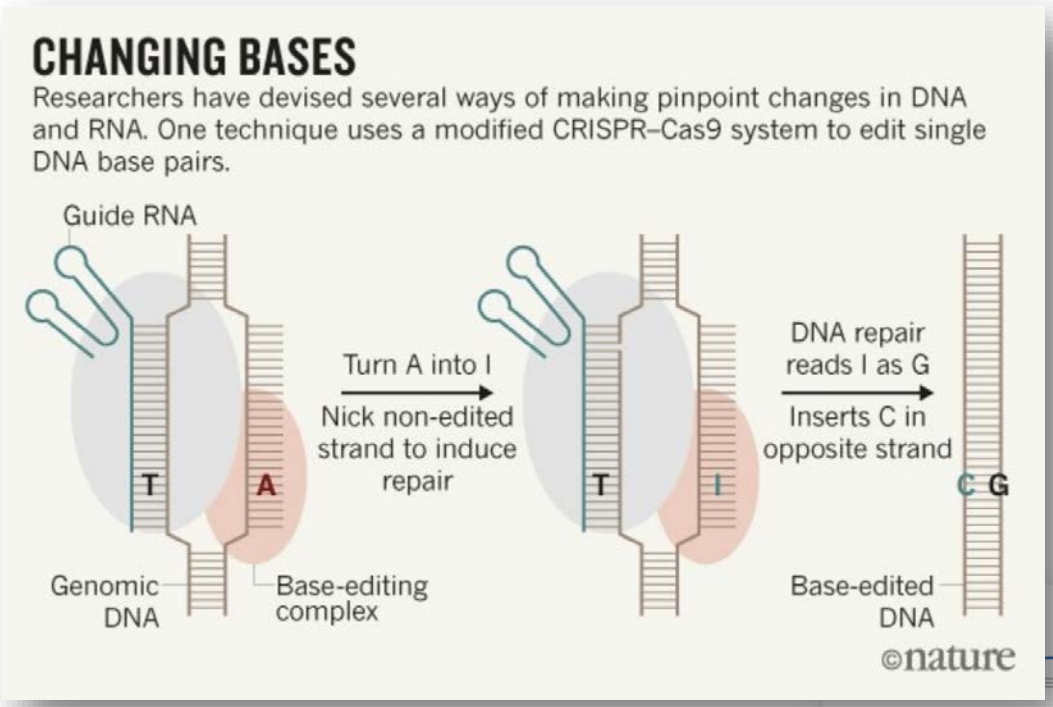
REPORT

Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9

Dong Niu[1,2,*], Hong-Jiang Wei[3,4,*], Lin Lin[5,*], Haydy George[1,*], Tao Wang[1,*], I-Hsiu Lee[1,*], Hong-Ye Zhao[3], Yong Wang[6], Yinan Kan[1], Ellen Shrock[7], Emal Lesha[1], Gang Wang[1], Yonglun Luo[5], Yubo Qing[3,4], Deling Jiao[3,4], Heng Zhao[3,4], Xiaoyang Zhou[6], Shouqi Wang[8], Hong Wei[6], Marc Güell[1,†], George M. Church[1,7,9,†], Luhan Yang[1,†,‡]

[1]eGenesis, Inc., Cambridge, MA 02139, USA.

CRISPR 2.0: Base Editing



doi:10.1038/nature24644

Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage

Nicole M. Gaudelli[1,2,3], Alexis C. Komor[1,2,3], Holly A. Rees[1,2,3], Michael S. Packer[1,2,3], Ahmed H. Badran[1,2,3], David I. Bryson[1,2,3] & David R. Liu[1,2,3]

Is CRISPR Safe?

Confirmatory Results

No unexpected CRISPR-Cas9 off-target activity gene-edited mice

Vivek Iyer, Katharina Boroviak, Mark Thomas, Brendan Doe, Edw

doi: https://doi.org/10.1101/263129

Abstract

CRISPR-Cas technologies have transformed genome-editing of experimental organisms and have immense therapeutic potential. Despite significant advances in our understanding of the CRISPR-Cas9 system, concerns remain over the potential for off-target effects. Recent studies have addressed these concerns using whole-genome sequencing (WGS) of gene-edited embryos or animals to search for de novo mutations (DNMs), which may represent candidate changes induced by poor editing fidelity. Critically, these studies used strain-matched but not pedigree-matched controls and thus were unable to reliably distinguish generational or colony-related differences from true DNMs. Here we used a trio design and whole genome sequenced 8 parents and 19 embryos, where 10 of the embryos were mutagenised with well-characterised gRNAs targeting the coat colour Tyrosinase (Tyr) locus. Detailed analyses of these whole genome data allowed us to conclude that if CRISPR mutagenesis were causing SNV or indel off-target mutations in treated embryos, then the number of these mutations is not statistically distinguishable from the background rate of DNMs occurring due to other processes.

New Results

Identification of Pre-Existing Adaptive Immunity to Cas9 Proteins in Humans

Carsten Trevor Charlesworth, Priyanka S Deshpande, Daniel P Dever, Beruh Dejene, Natalia Gomez-Ospina, Sruthi Mantri, Mara Pavel-Dinu, Joab Camarena, Kenneth I Weinberg, Matthew H Porteus

doi: https://doi.org/10.1101/243345

Abstract

The CRISPR-Cas9 system has proven to be a powerf
for the precise modification of specific DNA sequen
currently underway to use the CRISPR-Cas9 system
human genetic diseases. The most widely used hon

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IN THE LAB

CRISPR hits a snag: Our immune systems may attack the treatment

By ANDREW JOSEPH @DrewQJoseph / JANUARY 8, 2018

Uh Oh—CRISPR Might Not Work in People

A sampling of human blood has turned up a surprise: most people could be immune to one of the world's biggest advances in genetic engineering.

nature biotechnology

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley

"This is the first systematic assessment of unexpected events resulting from CRISPR/Cas9 editing in therapeutically relevant cells, and we found that changes in the DNA have been seriously underestimated before now. It is important that anyone thinking of using this technology for gene therapy proceeds with caution, and looks very carefully to check for possible harmful effects."

Allan Bradley
Director Emeritus
Wellcome Sanger Institute

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

Genome edit
the heterozy
accuracy an
response. In
homologous
the DSB was
embryos car
safety of the
embryos by
applications,

Shoukrat Mitalipov (OHSU)

50p

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i

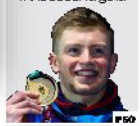
The essential daily briefing

THURSDAY
27 JULY 2017

i News.co.uk

Twin Peaty

Swimming hero wins second gold



i world exclusive

One giant step for designer babies

» **Revealed** Era of genetically modified babies moves closer, as scientists prove they can safely alter human embryos

» Inherited diseases caused by defective genes can be corrected in the earliest stage of life, revolutionary technique shows

» Same technology could be used to select stronger muscles or better eyesight, prompting fierce ethical debate

» 'They've done it. The quality of the work is high,' top scientist tells i

» Religious organisations likely to oppose groundbreaking research

SPECIAL REPORT BY STEVE CONNOR, PAGES 6-7

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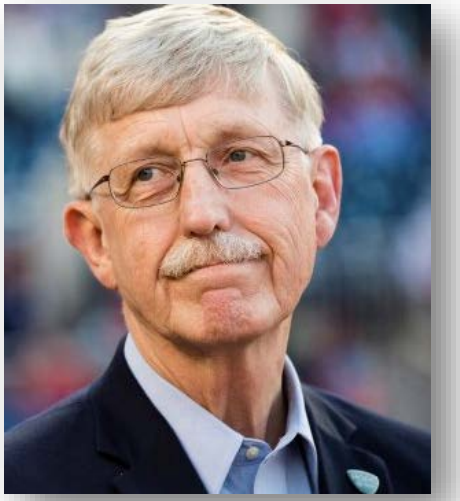
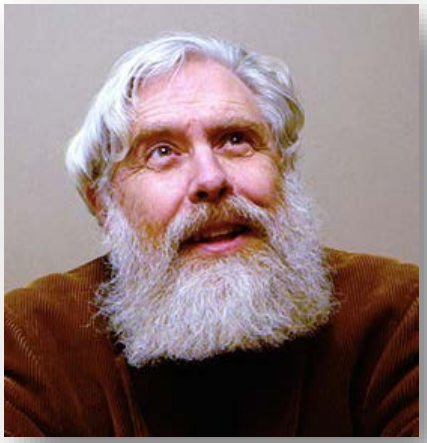
"The concept of altering the human germline in embryos for clinical purposes has been debated over many years from many different perspectives, and has been viewed almost universally as a line that should not be crossed… Designer babies make great Hollywood movies. They make really bad science, and I think they are really bad ethics."





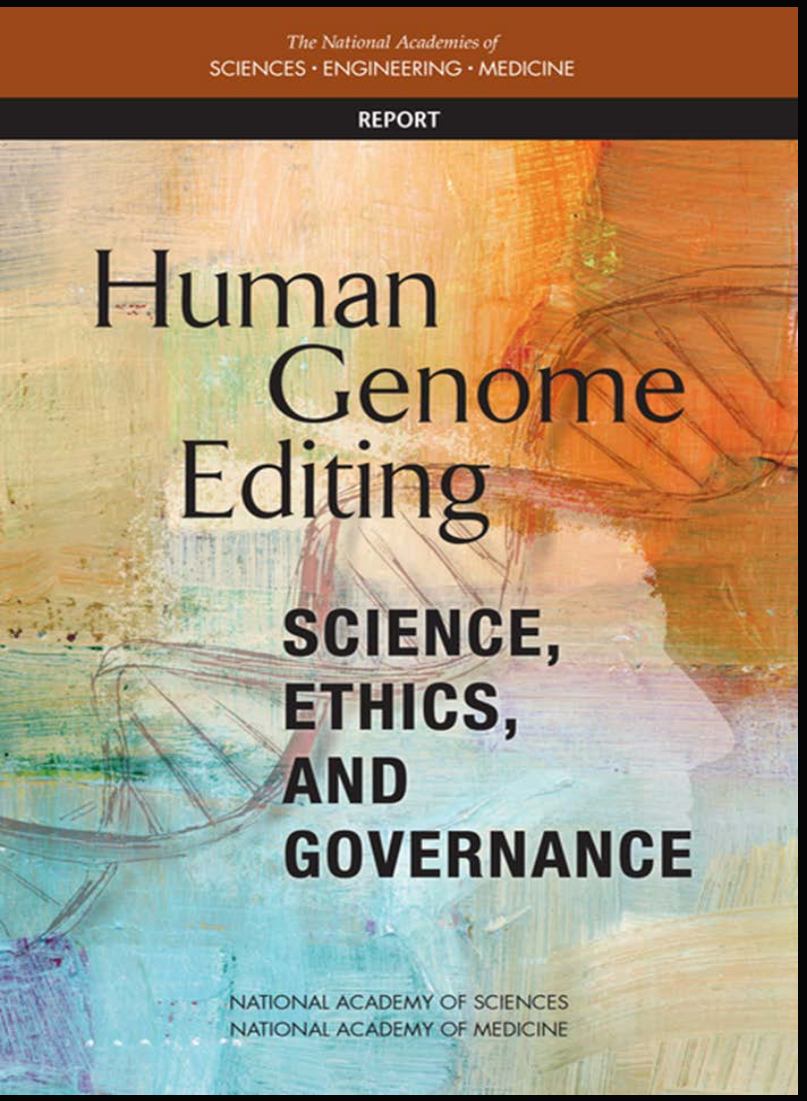
"Gene editing is already used to make GM-persons [cancer, HIV]… If by GM-persons, we limit our meaning to heritable DNA changes, then the most likely use would be changing deadly DNA variants into their common healthy versions. This should be far safer than testing new drugs, which impact complex human systems in unknown ways."

Tuesday, January 23, 2018

NIH to launch genome editing research program

Somatic Cell Genome Editing aims to develop tools for safe and effective genome editing in humans.

The National Institutes of Health will launch an effort aimed at removing barriers that slow the adoption of genome editing for treating patients. This program, Somatic Cell Genome Editing, plans to award researchers approximately $190 million over six years beginning this year, pending availability of funds. These researchers will collaborate to improve the delivery mechanisms for targeting gene editing tools in patients, develop new and improved genome editors, develop assays for testing the safety and efficacy of the genome editing tools in animal and human cells, and assemble a genome editing toolkit containing the resulting knowledge, methods, and tools to be shared with the scientific community. The program is funded by NIH's Common Fund.

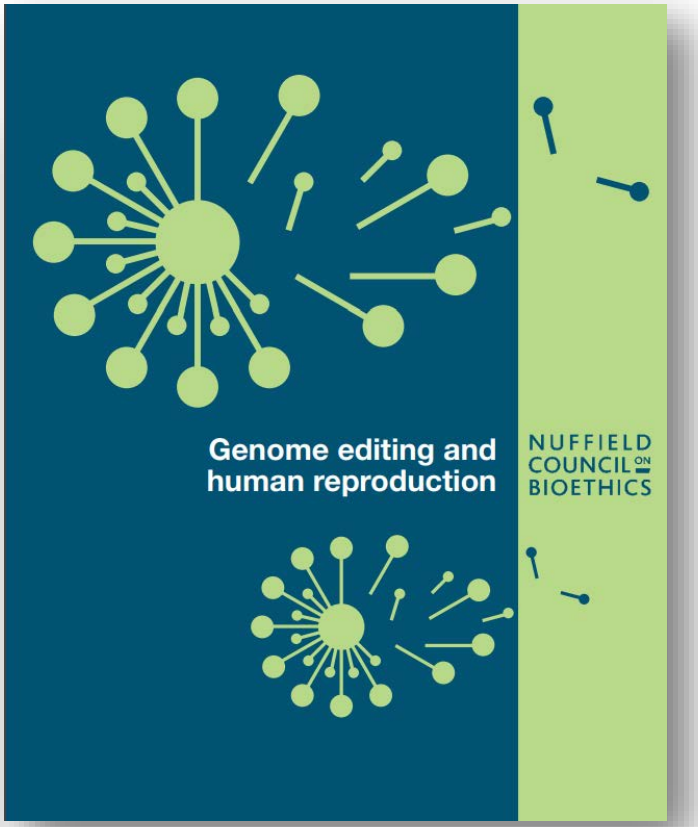


"We say proceed with all due caution, but we don't prohibit germline, after considerable discussion and debate. We're talking only about fixing diseases."

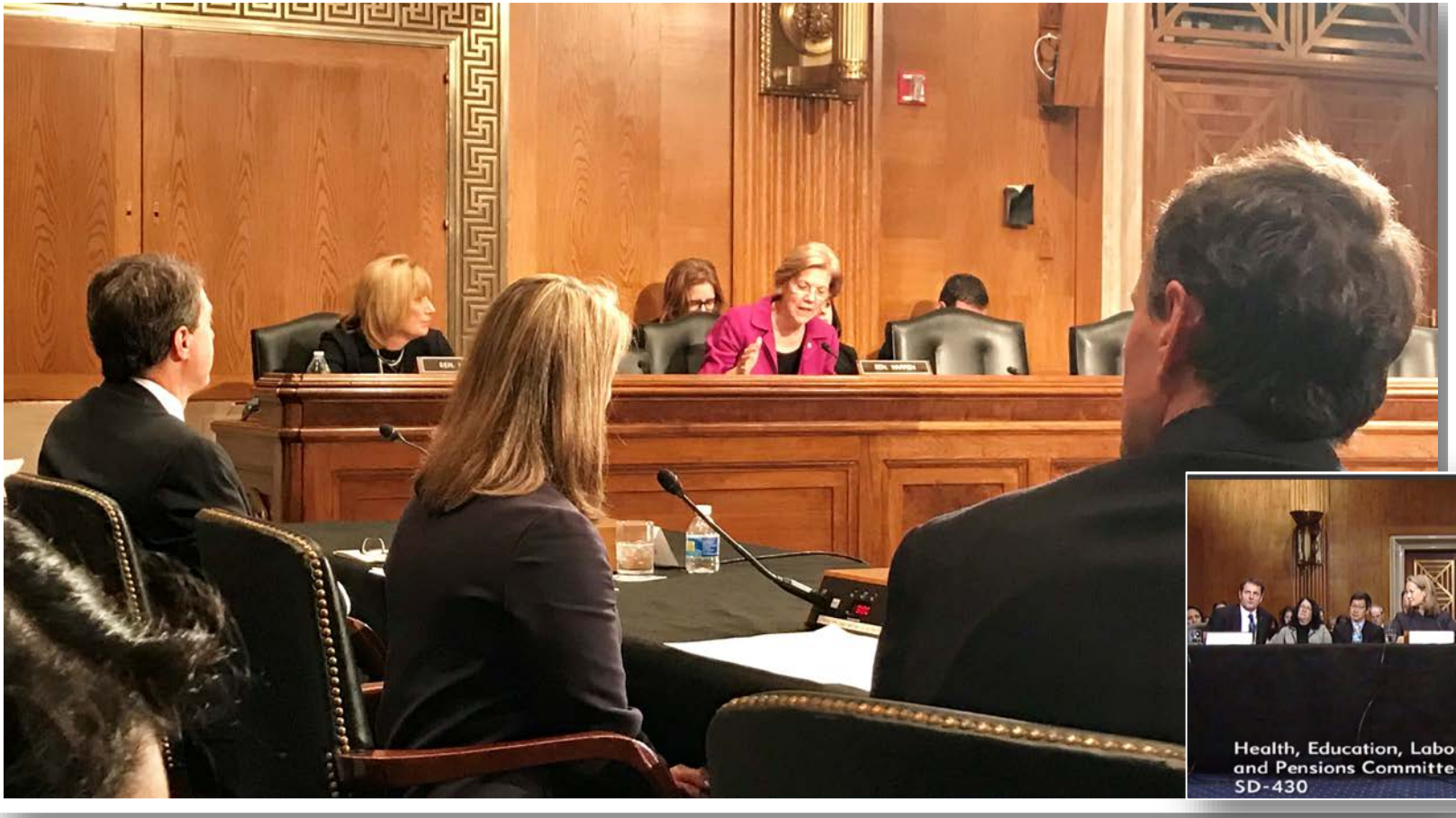
-- Rick Hynes (HHMI/MIT)

Genetically modified babies given go ahead by UK ethics body

The Nuffield Council on Bioethics says changing the DNA of a human embryo could be 'morally permissible' if it is in the child's best interests



CRISPR Goes to Washington



What Is a Genetically Modified Crop? A European Ruling Sows Confusion

In Europe, plants created with gene-editing technologies will be stringently regulated as G.M.O.'s. But older crops whose DNA has been altered will be left alone.

Matt Ridley @mattwridley — Follow

Catastrophic decision of the ECJ to go against the advice of its advocate general and treat gene-edited crops as GMOs.

Will ensure European farming is less competitive, less innovative, more dependent on chemicals and worse for the environment.

Ewan Birney @ewanbirney — Follow

So placing CRISPR in the same bucket of all GMO and uv, gamma, chemical mutations in another bucket is like the Catholic Church classifying ducks as fish

Jonathan Pettitt @genotripe — Follow

#ECJ statement is beyond dumb: "organisms obtained by mutagenesis are GMOs within the meaning of the GMO Directive".
All plants are GMOs by this definition - mutagenesis is an unavoidable consequence of DNA replication. #GMOs #CRISPR

Owen Paterson MP @OwenPaterson — Follow

All too predictable ruling from the European Court which sadly "threatens research on gene-edited crops in the bloc", & will see investment move elsewhere as the EU condemns itself to become the Museum of World Farming nature.com/articles/d4158 …

Kevin Folta @kevinfolta — Follow

Hey EU! Politicians have decided you won't get gene edited crops. Fire up the Cesium 137 source and order some ethyl methanesulfonate - you're doing it old school. Condolences to EU colleagues & farmers that can't benefit from the best technology.

Mark Lynas @mark_lynas — Follow

European court plumbs the depths of scientific absurdity with today's gene editing decision - random mutagenesis is OK, while precision editing is a 'GMO' and therefore borderline illegal. Go figure! Like saying doctors can use blunderbuss but not scalpel.

Clive G. Brown @Clive_G_Brown — Follow

Central also is the misanthropic idea that there is a 'Nature' that exists in a state of perfected harmony and that humans and all their works are corrupting it.
If only these twits realized that all of their beloved vegetables and most farm animals are hideous mutants.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- **S**peed of peer review and production
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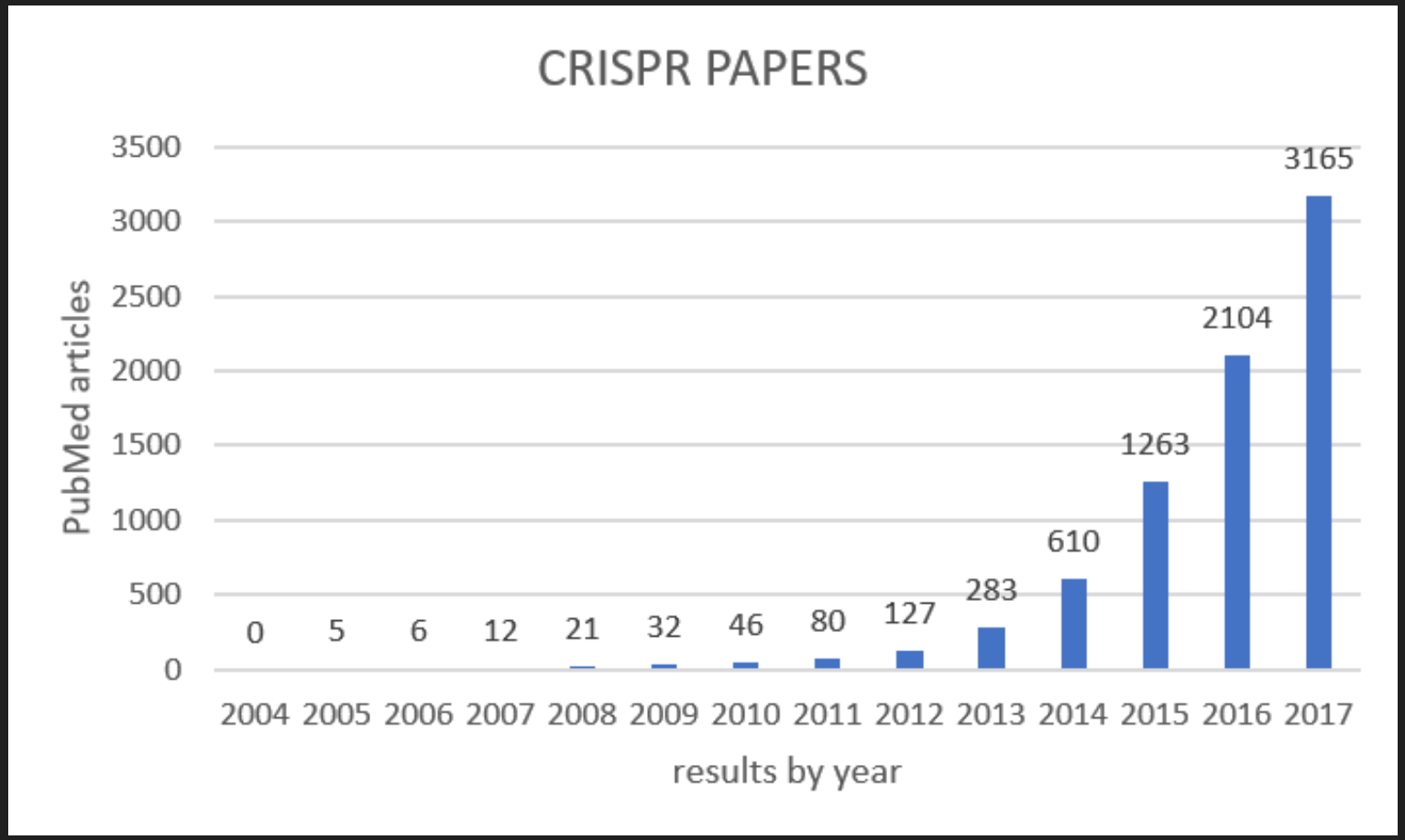
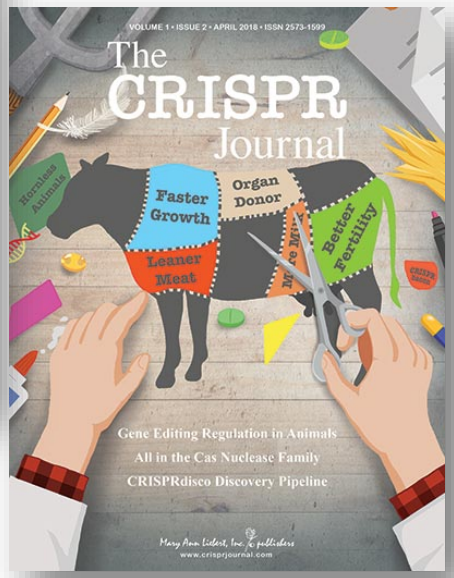


The Story So Far…

FEBRUARY



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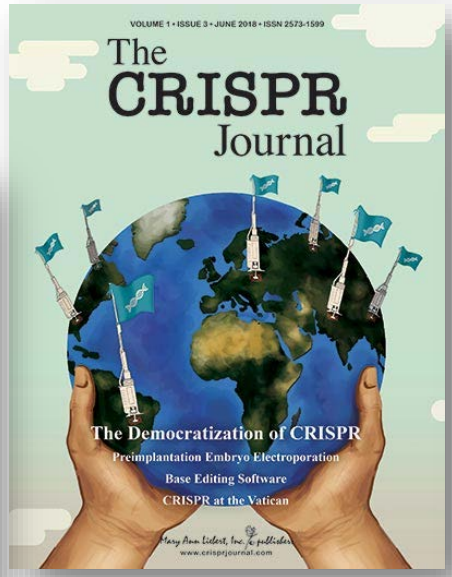

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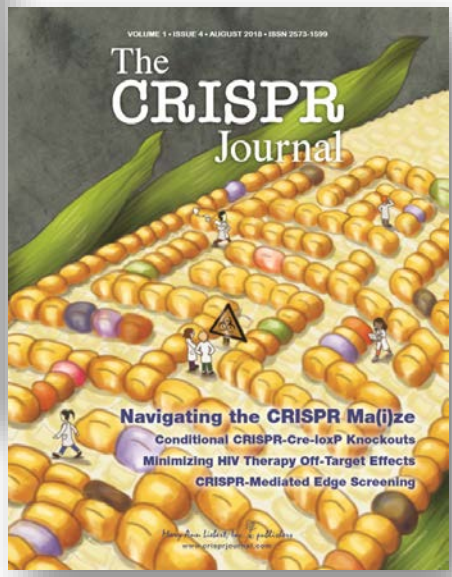
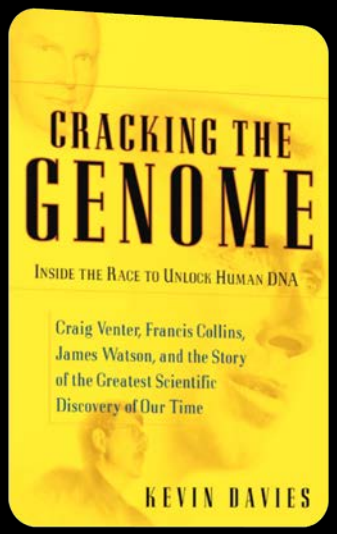
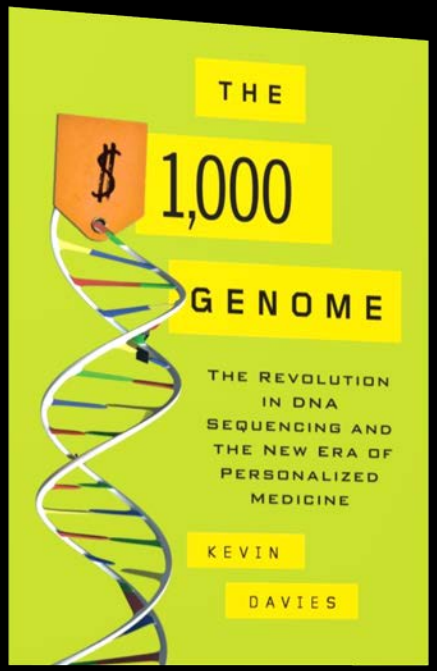


AUGUST



2001



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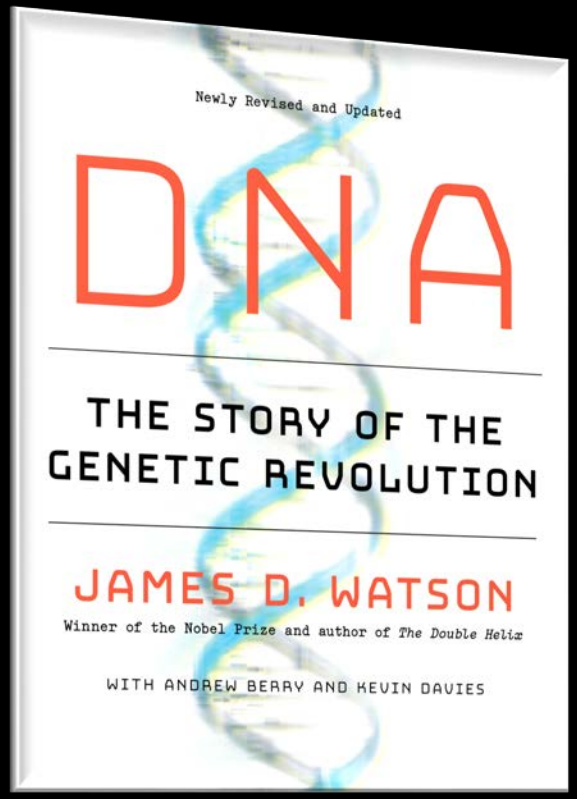

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