KICK OFF MEETING

International Association for Responsible Research and Inovation in Genome Editing (ARRIGE)

FRIDAY MARCH 23rd, 2018
PARIS

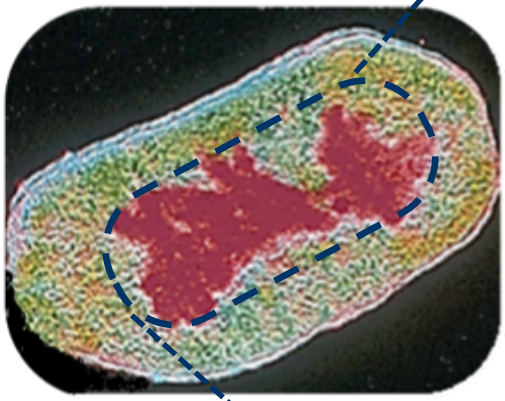
Setting the stage, where do we stand today with CRISPR technology

Francisco J.M. Mojica

Universitat d'Alacant
Universidad de Alicante

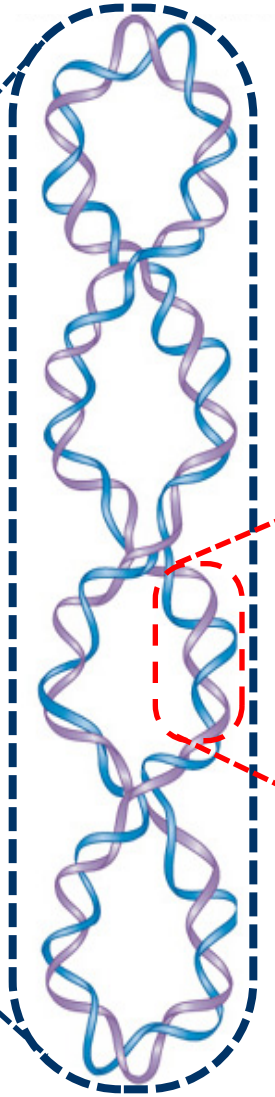
Plain CRISPR

Prokaryotes



The MacGraw-Hill companies.
Microbiology, by Precott , Harley & Klein. 7th Ed.

Genome



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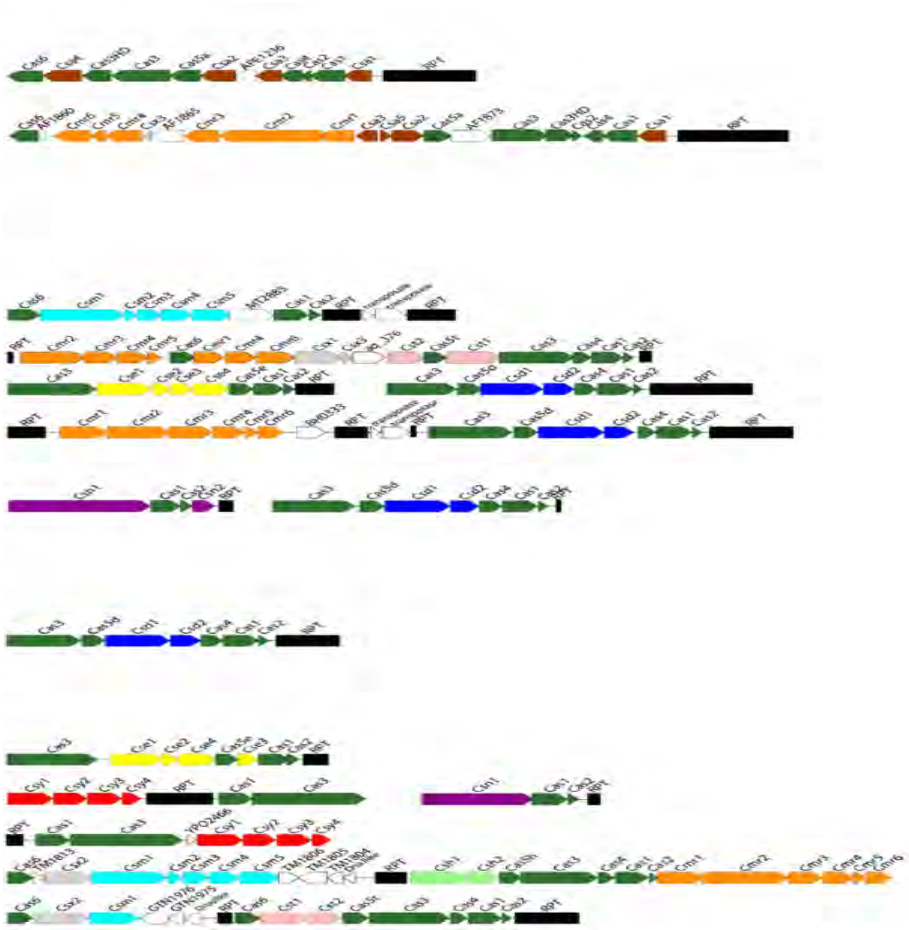
GTTACAGACGAACCCTAGTTGGGTTGAAGCCACGACAATCAAGTCTGGTTGCATGGCGACACG
GAGTTACAGACGAACCCTAGTTGGGTTGAAGCCTGTGCCTCCAGCGGCCGTCAGACAGTCGCA
TCCGAGTTACAGACGAACCCTAGTTGGGTTGAAGCAAGAAGCCGCTCGCCGTCCTCGATGACG
GGCGGGCGGTTACAGACGAACCCTAGTTGGGTTGAAGCGACAAGACTCGCGACGAAGCCGAGT
CGAAACGCCGCGTTACAGACGAACCCTAGTTGGGTTGAAGCCTCTTTATCCCTCCTGCCCGAA
TGTCTACGAATATCGTTACAGACGAACCCTAGTTGGGTTGAAGCGAACCCACTGGTGAAGAAA
AAGTTGTAGAGACCCTAGTTACAGACGAATCCCTAGTTGGGTTGAAGCACGACAATCAAGTCT
GGTTACATGGCGACAGGATGGGTTACAGACGAACCCTAGTTGGGTTGAAGCTTCCACAACGTC
GGGGAGGGCGAAATTAGCCAAGCAGTTACAGACGAACCCTAGTTGGGTTGAAGC

Spacers

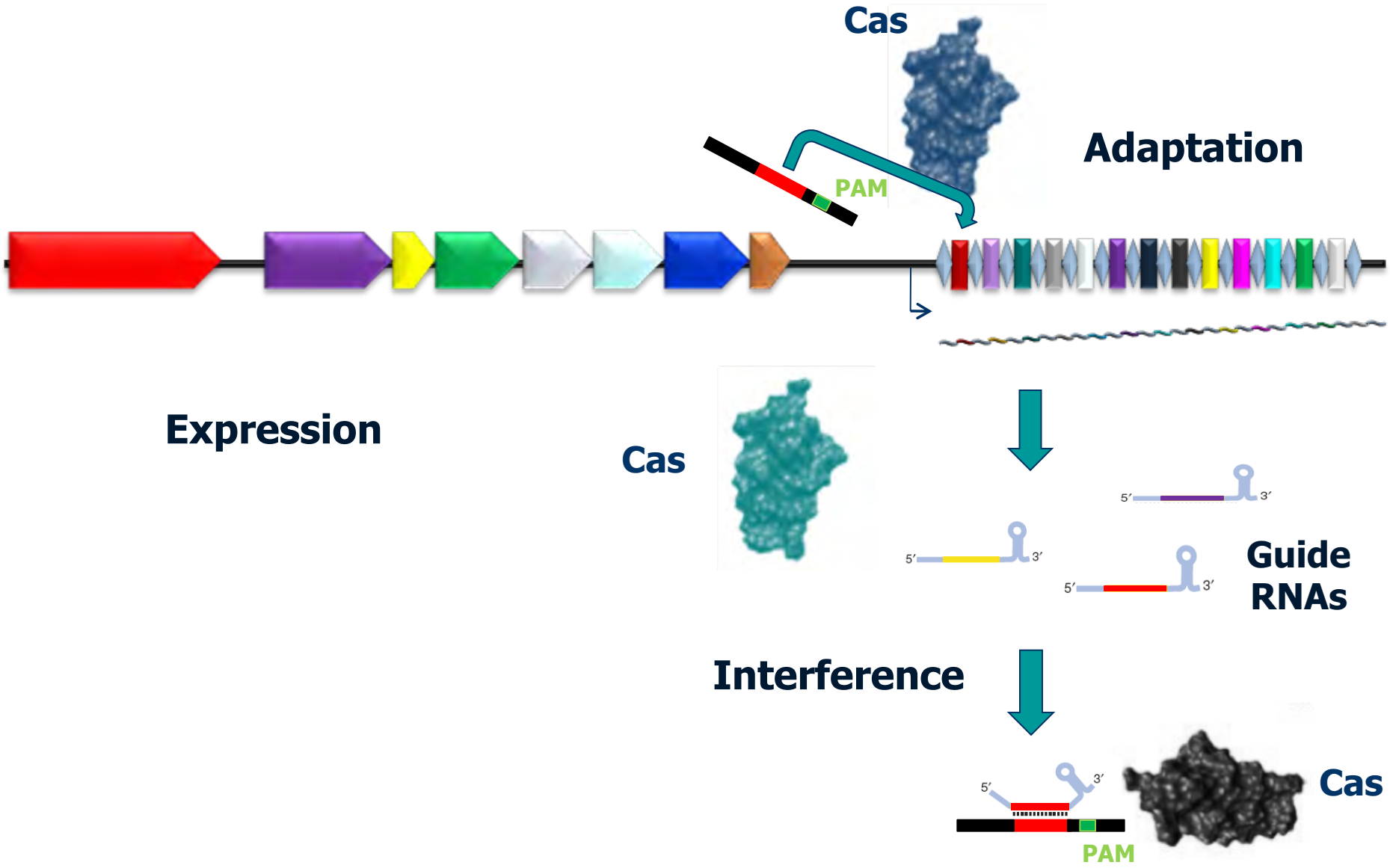
cas (***C****RISPR* ***as****sociated*)

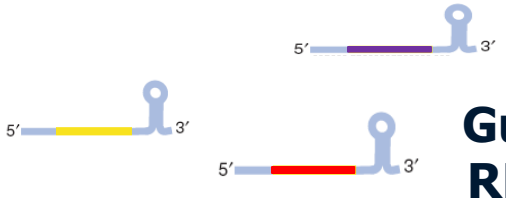


Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeat

Modified from Haft et al. PLoS Comput. Biol. 2005

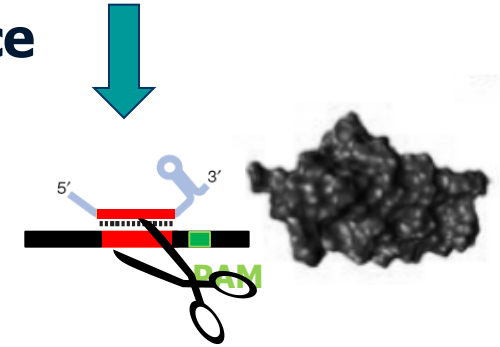
The CRISPR Mechanism


Cas
PAM
Adaptation
Expression
Cas
5′
3′
5′
3′
5′
3′
Guide
RNAs
Interference
5′
3′
PAM
Cas



Guide RNAs

Interference



Cas

Programmable DNA cleavage

PNAS

Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria

Giedrius Gasiunas[a], Rodolphe Barrangou[b], Philippe Horvath[c], and Virginijus Siksnys[a,1]

[a]Institute of Biotechnology, Vilnius University, LT-02241 Vilnius, Lithuania; [b]DuPont Nutrition and Health, Madison, WI 53716; and [c]DuPont Nutrition and Health, F-86220 Dangé-Saint-Romain, France

Edited by Arthur Landy, Brown University, Providence, RI, and approved August 1, 2012 (received for review May 21, 2012)

R. Barrangou

P. Horvath

V. Siksnys

Science

AAAS

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

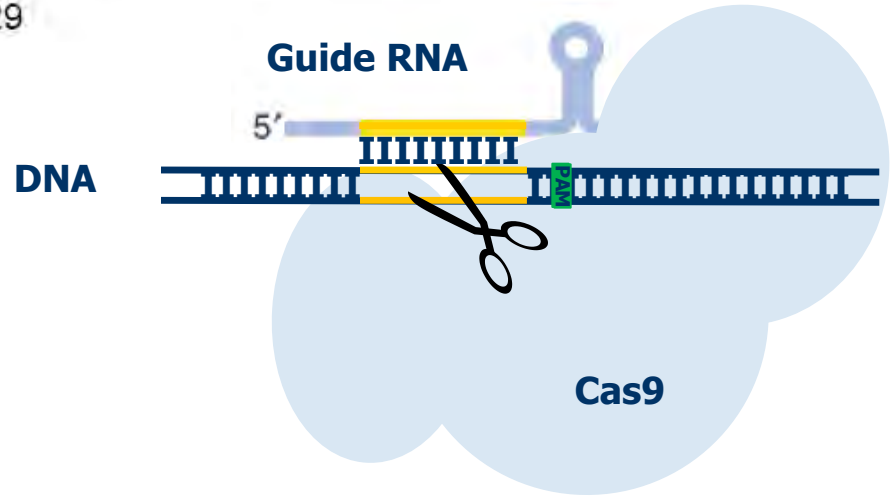
Martin Jinek *et al.*

Science **337**, 816 (2012);
DOI: 10.1126/science.1225829

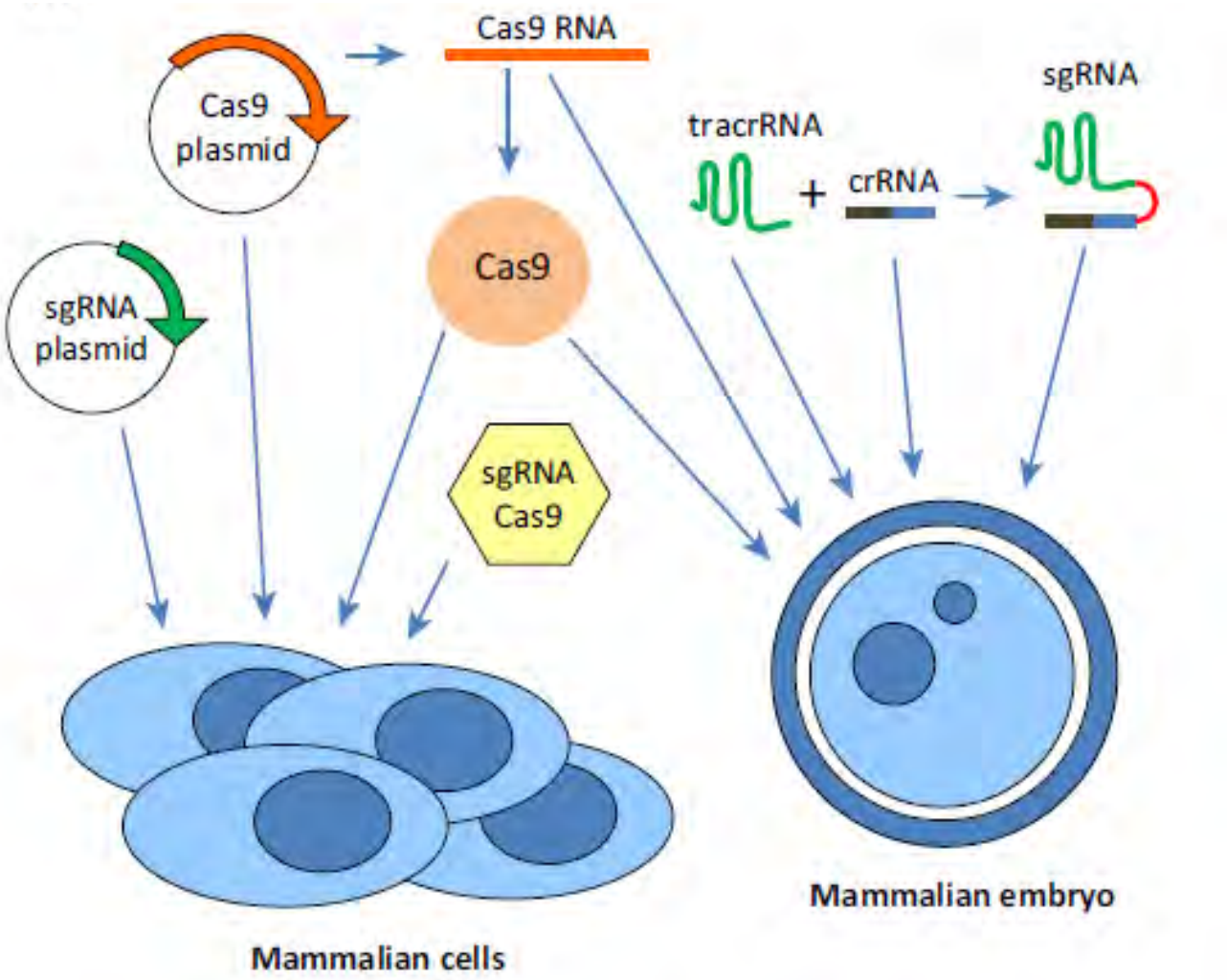
"... to exploit the system for RNA-programmable genome editing"

E. Charpentier

J. Doudna



CRISPR-Cas9 delivery



Trends in Microbiology

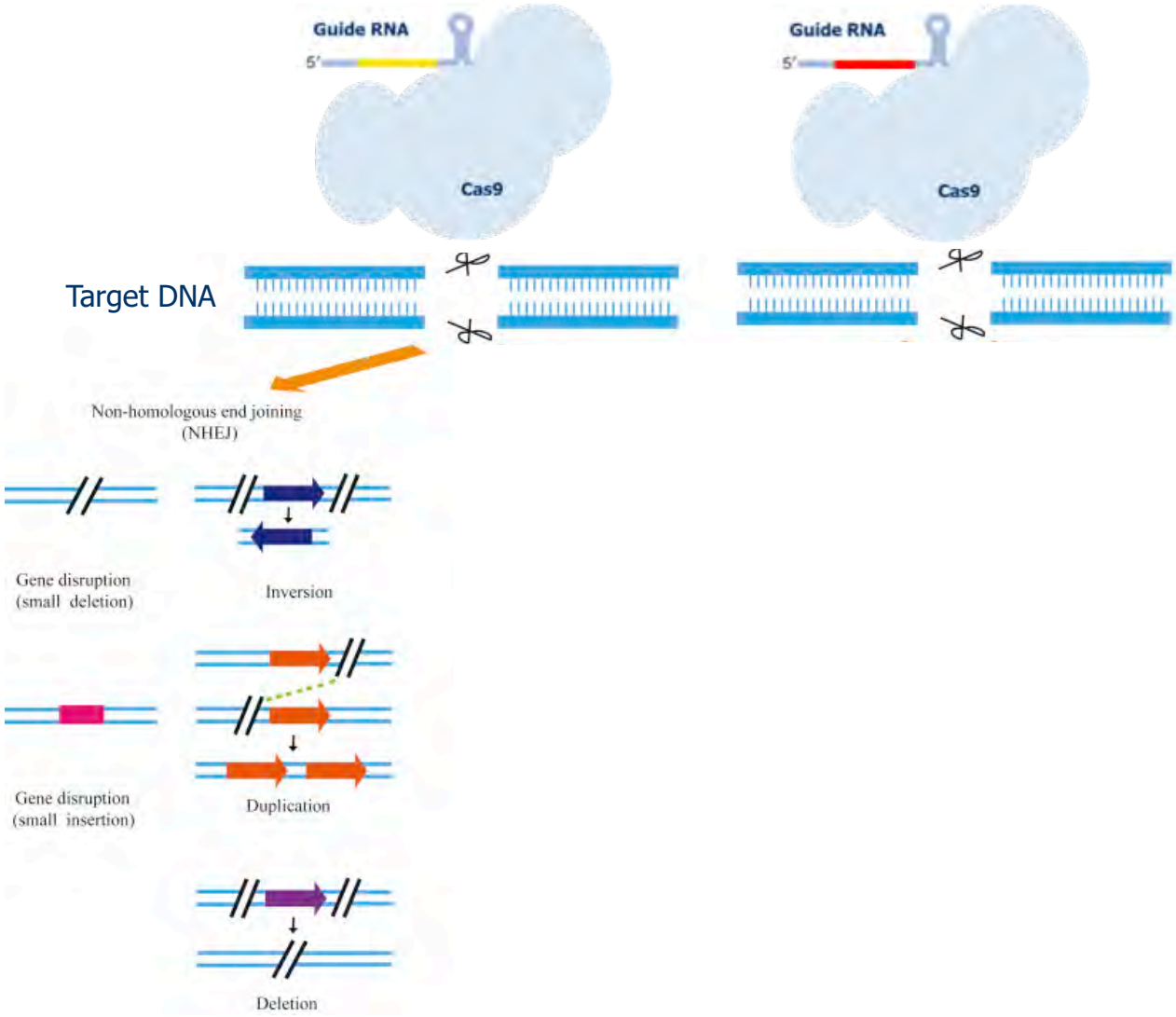
Review

On the Origin of CRISPR-Cas Technology: From Prokaryotes to Mammals

Francisco J.M. Mojica[1,2] and Lluis Montoliu[3,4,*]

Genome Editing

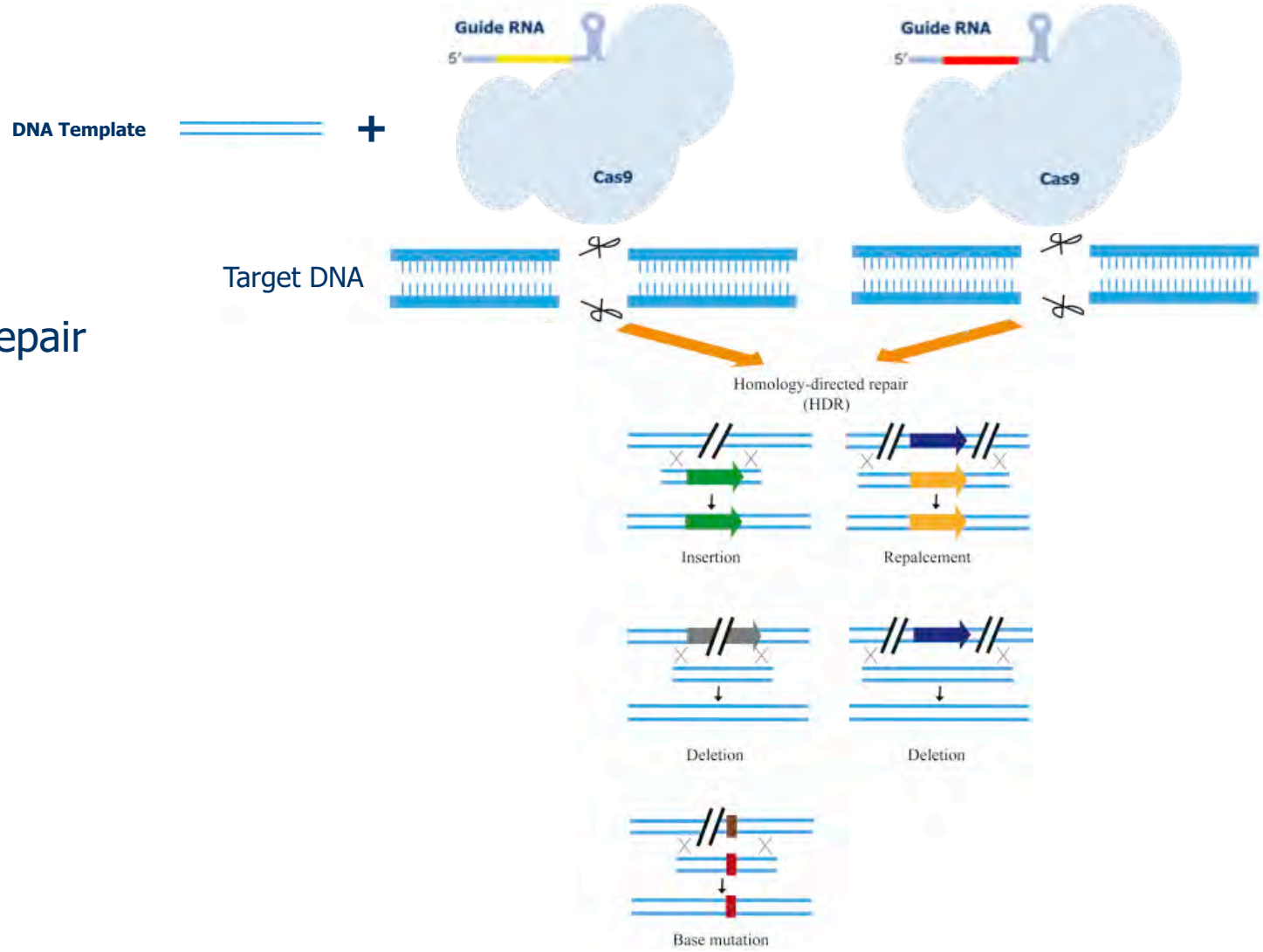
DNA Repair



Yue Mei, et al. Journal of Genetics and Genomics, Volume 43, Issue 2, 2016, 63–75

Genome Editing

DNA Repair



Yue Mei, et al. Journal of Genetics and Genomics, Volume 43, Issue 2, 2016, 63–75

Genome Editing

Science. 2013 February 15; 339(6121): 819–823. doi:10.1126/science.1231143.

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], and **Feng Zhang**[1,†]

L. Marraffimi

F. Zhang

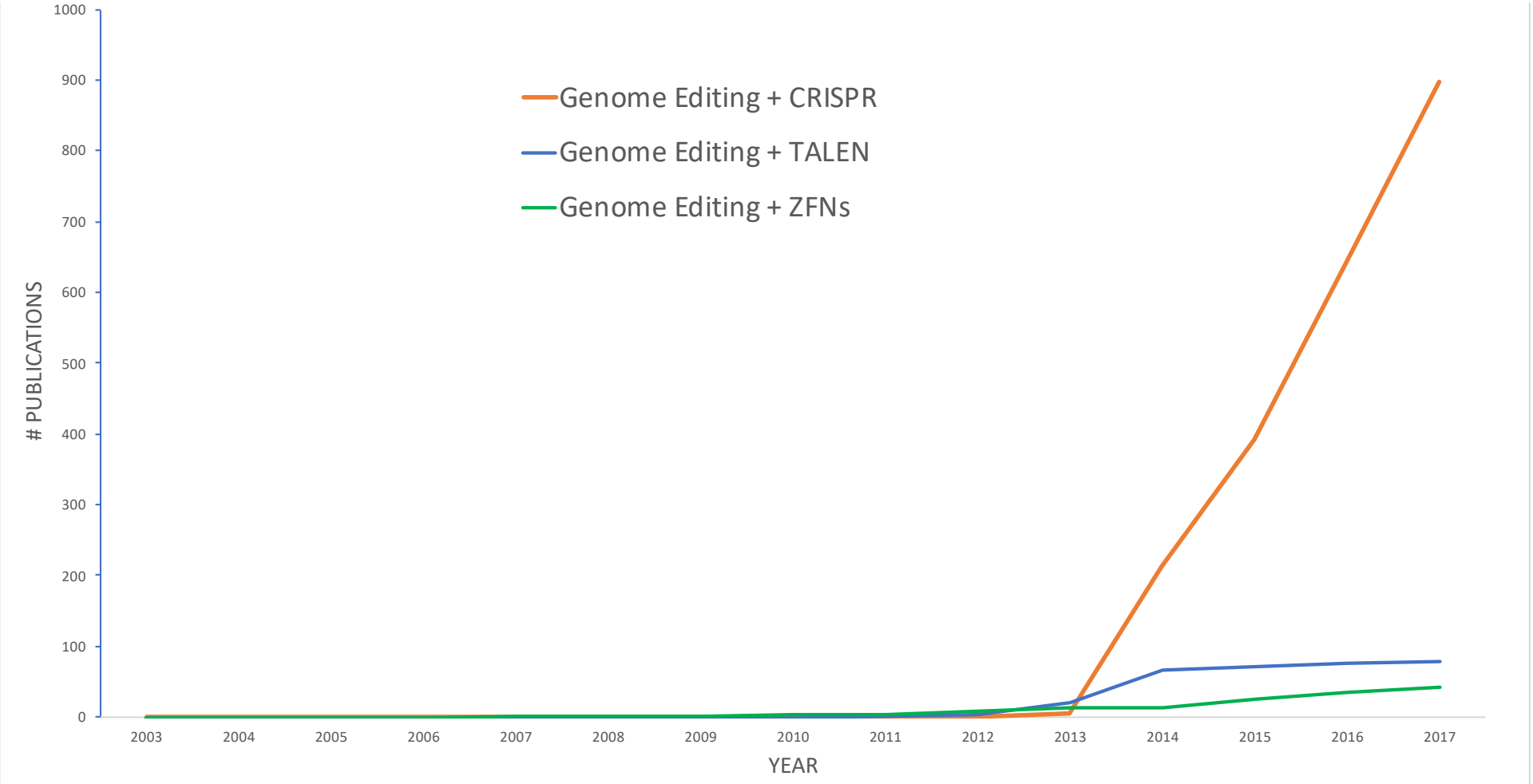
Science. 2013 February 15; 339(6121): 823–826. doi:10.1126/science.1232033.

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali[1,5], **Luhan Yang**[1,3,5], **Kevin M. Esvelt**[2], **John Aach**[1], **Marc Guell**[1], **James E. DiCarlo**[4], **Julie E. Norville**[1], and **George M. Church**[1,2,*]

G. Church

TALENs , ZFN *et al*. Superseded by CRISPR



nature International weekly journal of science

NATURE | **LETTER**

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Alexis C. Komor, Yongjoo B. Kim, Michael S. Packer, John A. Zuris & David R. Liu

Affiliations | **Contributions** | **Corresponding author**

Nature (2016) | doi:10.1038/nature17946

Received 26 February 2016 | Accepted 30 March 2016 | Published online 20 April 2016

"CRISPR-edited" organisms

- **Bacteria**
- **Protozoa***: Leishmania, Toxoplasma, Plasmodium, Trypanosoma*…
- **Fungi:** yeasts, molds, mushroom
- **Plants**:

Rice	Orange	Wheat
Soybean	Corn	Potato
Tomato	Tobacco	*Arabidopsis*
Grapefruit	Petunia	Cotton
Lettuce	Cucumber	Sorghum

"CRISPR-edited" organisms

- **Worms**
- **Insects**
- **Reptiles**
- **Amphibians**
- **Molluscs**
- **Fish**
- **Birds**
- **Mammals**: mouse, rabbit, goat, rat, dog, pig, primate…

Targeting Disease


DAILY NEWS 15 February 2018

CRISPR has fixed the genetic cause of a learning disability

Fragile X syndrome is the most common genetic cause of intellectual disability
Christine Harrison/Visuals Unlimited, Inc. /SCIENCE PHOTO LIBRARY

Cancer
Malaria
Albinism
Cataracts
Hemophilia
Cystic fibrosis
β-thalassemia
Retinitis Pigmentosa
Hypercholestorolemia
Neurodegenerative disorders
Duchenne Muscular Dystrophy
Viral infections (HV, HBV, HPV, HIV…

Editing Human Embryos

Protein Cell
DOI 10.1007/s13238-015-0153-5

Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

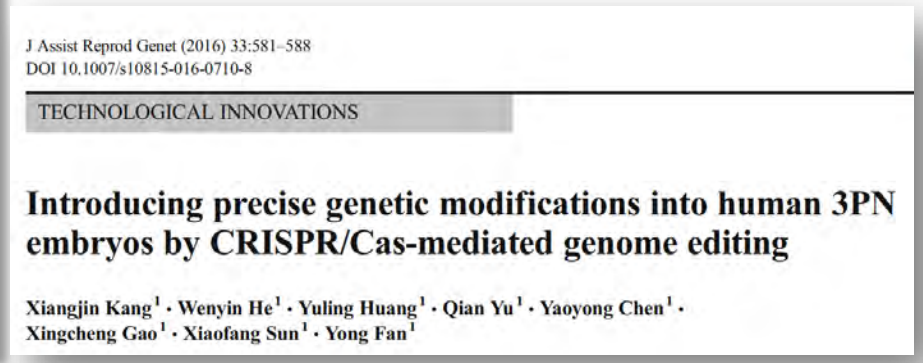
Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou, Junjiu Huang

Mol Genet Genomics (2017) 292:525–533
DOI 10.1007/s00438-017-1299-z

ORIGINAL ARTICLE

CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein

Lichun Tang[1,2] · Yanting Zeng[3] · Hongzi Du[3] · Mengmeng Gong[1] · Jin Peng[1] · Buxi Zhang[1] · Ming Lei[3] · Fang Zhao[4] · Weihua Wang[5] · Xiaowei Li[6] · Jianqiao Liu[3]



J Assist Reprod Genet (2016) 33:581–588
DOI 10.1007/s10815-016-0710-8

TECHNOLOGICAL INNOVATIONS

Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing

Xiangjin Kang[1] · Wenyin He[1] · Yuling Huang[1] · Qian Yu[1] · Yaoyong Chen[1] · Xingcheng Gao[1] · Xiaofang Sun[1] · Yong Fan[1]

ARTICLE

doi:10.1038/nature24033

Genome editing reveals a role for OCT4 in human embryogenesis

Norah M. E. Fogarty[1], Afshan McCarthy[1], Kirsten E. Snijders[2], Benjamin E. Powell[3], Nada Kubikova[4], Paul Blakeley[1], Rebecca Lea[1], Kay Elder[5], Sissy E. Wamaitha[1], Daesik Kim[6], Valdone Maciulyte[3], Jens Kleinjung[7], Jin-Soo Kim[6,8], Dagan Wells[4], Ludovic Vallier[2,9,10], Alessandro Bertero[10]†, James M. A. Turner[3] & Kathy K. Niakan[1]

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]§

Gene Ther. 2016 May 19. doi: 10.1038/gt.2016.41. [Epub ahead of print]

Excision of HIV-1 DNA by gene editing: a proof-of-concept in vivo study.

Kaminski R[1], Bella R[2], Yin C[1], Otte J[1], Ferrante P[2], Gendelman HE[3], Li H[4], Booze R[4], Gordon J[1], Hu W[1], Khalili K[1].

LETTER

doi:10.1038/nature25164

Treatment of autosomal dominant hearing loss by *in vivo* delivery of genome editing agents

Xue Gao[1,2,3]†*, Yong Tao[4,5]†*, Veronica Lamas[4], Mingqian Huang[4], Wei-Hsi Yeh[1,2,3,6], Bifeng Pan[7], Yu-Juan Hu[4,5], Johnny H. Hu[1,2,3], David B. Thompson[1,2], Yilai Shu[4,8], Yamin Li[9], Hongyang Wang[4,10], Shiming Yang[10], Qiaobing Xu[9], Daniel B. Polley[4], M. Charles Liberman[4], Wei-Jia Kong[5], Jeffrey R. Holt[7], Zheng-Yi Chen[4]§ & David R. Liu[1,2,3]§

Research Article

EMBO Molecular Medicine

CRISPR/Cas9-mediated somatic correction of a novel coagulator factor IX gene mutation ameliorates hemophilia in mouse

Yuting Guan[1,†], Yanlin Ma[2,3,†,*], Qi Li[2], Zhenliang Sun[4], Lie Ma[1], Lijuan Wu[1], Liren Wang[1], Li Zeng[1], Yanjiao Shao[1], Yuting Chen[1], ... Yu[3],

Sci Adv. 2017 Dec 20;3(12):eaar3952. doi: 10.1126/sciadv.aar3952. eCollection 2017 Dec.

In vivo genome editing improves motor function and extends survival in a mouse model of ALS.

Gaj T[1], Ojala DS[2], Ekman FK[3], Byrne LC[4], Limsirichai P[5], Schaffer DV[1,2,4].

ScienceDaily

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Science News *from research organizations*

New gene-editing technology partially restores vision in blind animals

Date: November 16, 2016

Source: Salk Institute

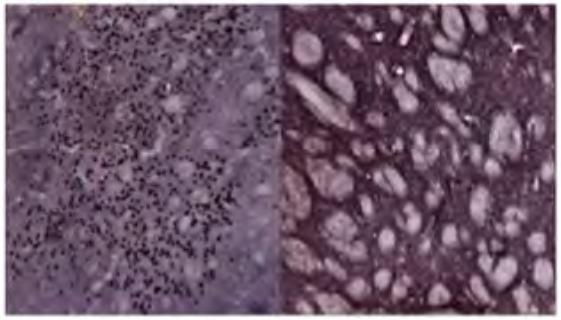
Summary: Researchers have discovered a holy grail of gene editing -- the ability to, for the first time, insert DNA at a target location into the non-dividing cells that make up the majority of adult organs and tissues. The technique, which the team showed was able to partially restore visual responses in blind rodents, will open new avenues for basic research and a variety of treatments, such as for retinal, heart and neurological diseases.

Science

AAAS

NEWS SCIENCE JOURNALS CAREERS MULTIMEDIA COLLECTIONS

LATEST NEWS



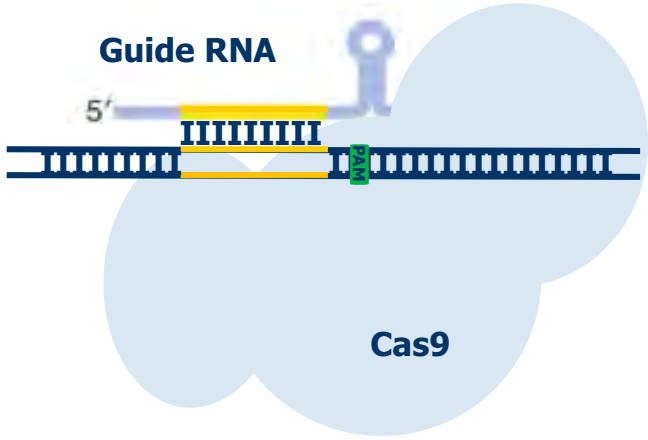
The brain of an untreated mouse on the left, showing huntingtin protein aggregation (a hallmark of Huntington's disease) and on the right, the brain of a mouse treated with CRISPR-Cas9 editing, showing the lack of protein aggregation.

Gene-editing method halts production of brain-destroying proteins

Clinical Trials with CRISPR

Row	Saved	Status	Study Title	Conditions	Interventions
1		Not yet recruiting	A Safety and Efficacy Study of TALEN and **CRISPR**/Cas9 in the Treatment of HPV-related Cervical Intraepithelial Neoplasial	• Human Papillomavirus-Related Malignant Neoplasm	• Biological: TALEN • Biological: **CRISPR**/Cas9
2		Recruiting	Safety of Transplantation of **CRISPR** CCR5 Modified CD34+ Cells in HIV-infected Subjects With Hematological Malignances	• HIV-1-infection	• Genetic: CCR5 gene modification
3		Not yet recruiting	Examining the Knowledge, Attitudes, and Beliefs of Sickle Cell Disease Patients, Parents of Patients With Sickle Cell Disease, and Providers Towards the Integration of **CRISPR** in Clinical Care	• Sickle Cell Disease	
4		Recruiting	NY-ESO-1-redirected **CRISPR** (TCRendo and PD1) Edited T Cells (NYCE T Cells)	• Multiple Myeloma • Melanoma • Synovial Sarcoma • Myxoid/Round Cell Liposarcoma	• Biological: NY-ESO-1 redirected autologous T cells with **CRISPR** edited endogenous TCR and PD-1 • Drug: Cyclophosphamide • Drug: Fludarabine • Device: NY-ESO-1 expression testing
5		Recruiting	Identification of Host Factors of Norovirus Infections in Mini-Gut Model	• Gastrointestinal Infection	• Procedure: Duodenal biopsy • Procedure: Saliva
6		Recruiting	A Study Evaluating UCART019 in Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma	• B Cell Leukemia • B Cell Lymphoma	• Biological: UCART019
7		Recruiting	A Feasibility and Safety Study of Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cell Immunotherapy for Relapsed or Refractory Leukemia and Lymphoma	• B Cell Leukemia • B Cell Lymphoma	• Biological: Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cells
8		Recruiting	PD-1 Knockout Engineered T Cells for Advanced Esophageal Cancer	• Esophageal Cancer	• Other: PD-1 Knockout T Cells

https://clinicaltrials.gov/ct2/results?cond=&term=crispr&cntry=&state=&city=&dist=



No PAM

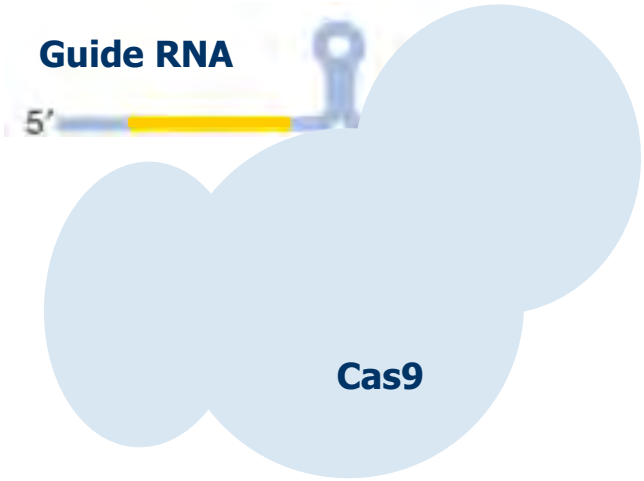
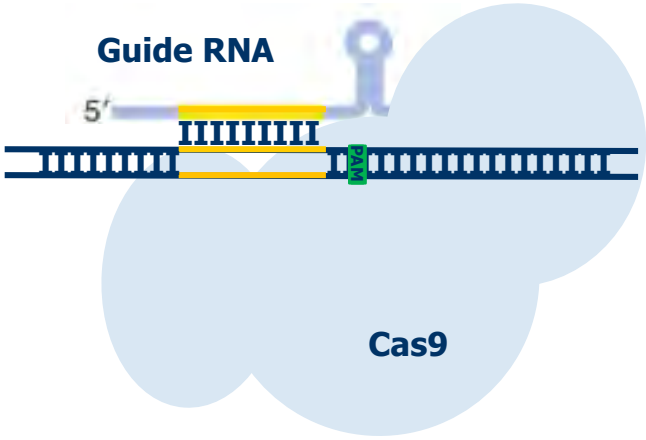


Table 2
Reported Cas9 variants and orthologues

Name	Origin	Primary PAM
SpCas9	*Streptococcus pyogenes*	NGG (3′ to protospacer)[a]
SpCas9-VQR	Engineered	NGAG (3′ to protospacer)
SpCas9-VRER	Engineered	NGCG (3′ to protospacer)
eSpCas9 (1.1)	Engineered	NGG (3′ to protospacer)
NmCas9	*Neisseria meningitidis*	NNNNGATT (3′ to protospacer)
St1Cas9	*Streptococcus thermophiles*	NNAGAA (3′ to protospacer)
SaCas9	*Staphylococcus aureus*	NNGGGT (3′ to protospacer)
AsCpf1	*Acidaminococcus sp. BV3L6*	TTTN (5′ to protospacer)
BlCas9	*Brevibacillus laterosporus*	NNNNCNAA (3′ to protospacer)

Zhang, D., et al., Journal of Genetics and Genomics (2016)



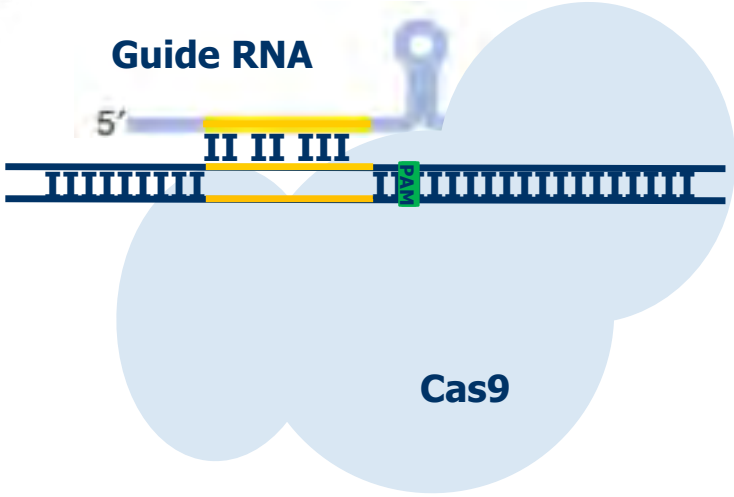
Off-Targets

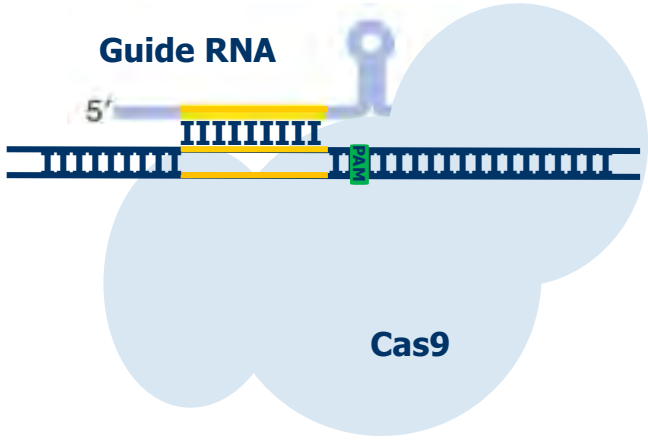
ARTICLE

doi:10.1038/nature16526

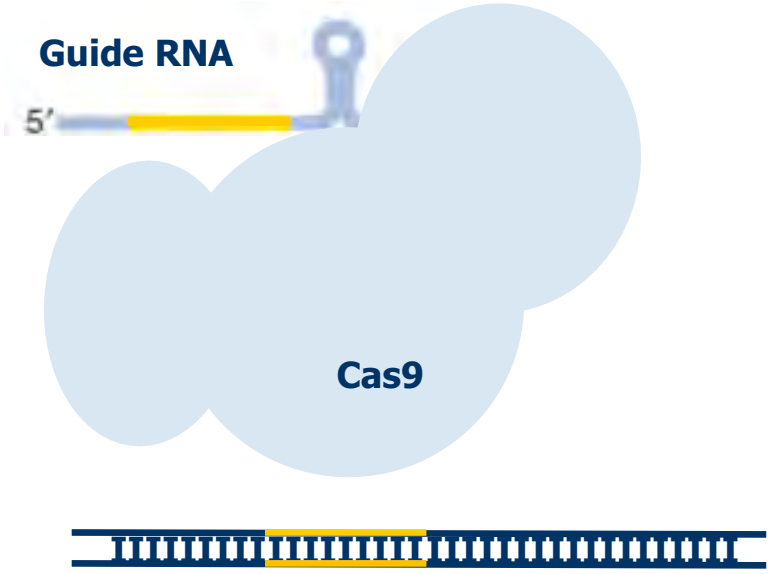
High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects

Benjamin P. Kleinstiver[1,2*], Vikram Pattanayak[1,2*], Michelle S. Prew[1], Shengdar Q. Tsai[1,2], Nhu T. Nguyen[1], Zongli Zheng[3] & J. Keith Joung[1,2]

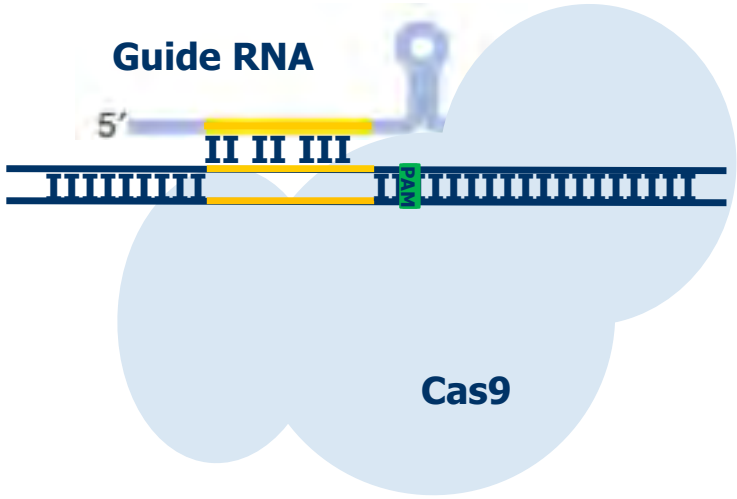




No PAM



Off-Targets



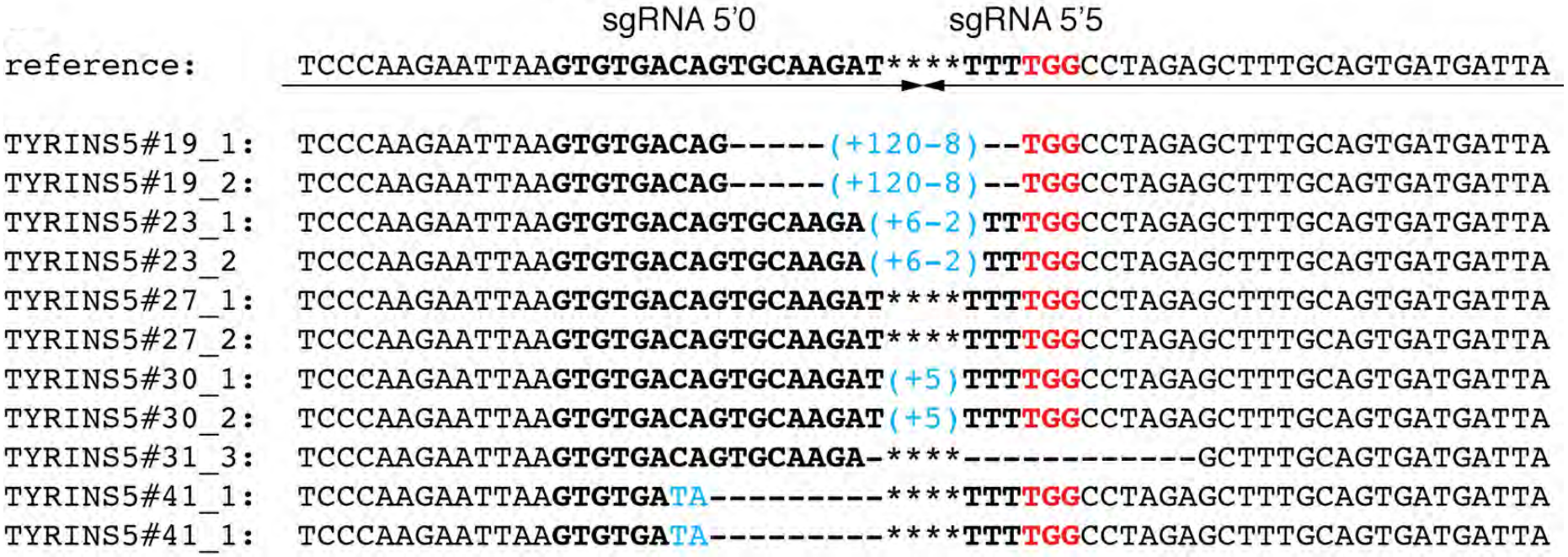
ARTICLE

doi:10.1038/nature26155

Evolved Cas9 variants with broad PAM compatibility and high DNA specificity

Johnny H. Hu[1,2,3], Shannon M. Miller[1,2,3], Maarten H. Geurts[1,2,3], Weixin Tang[1,2,3], Liwei Chen[1,2,3], Ning Sun[1,2,3], Christina M. Zeina[1,2,3], Xue Gao[1,2,3], Holly A. Rees[1,2,3], Zhi Lin[1,2,3] & David R. Liu[1,2,3]

On-Target Variations



Seruggia et al. 2015 NAR

Science

RESEARCH ARTICLES

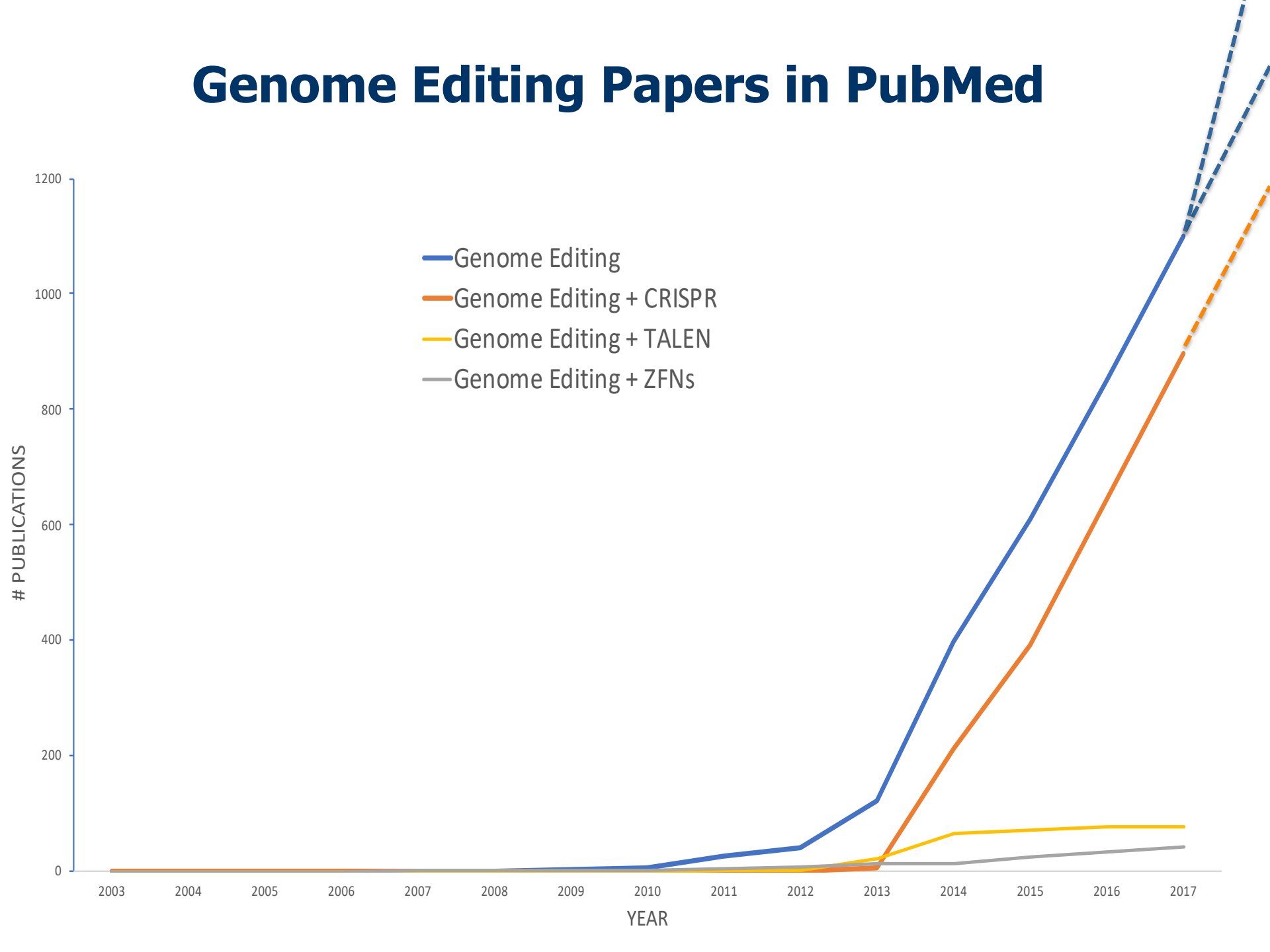
Cite as: D. B. T. Cox *et al.*, *Science*
10.1126/science.aaq0180 (2017).

RNA editing with CRISPR-Cas13

David B.T. Cox,[1,2,3,4,5,6*] Jonathan S. Gootenberg,[1,2,3,4,7*] Omar O. Abudayyeh,[1,2,3,4,6*] Brian Franklin,[1,2,3,4] Max J. Kellner,[1,2,3,4] Julia Joung,[1,2,3,4] Feng Zhang[1,2,3,4†]

Feng Zhang

Genome Editing Papers in PubMed



Science

RESEARCH ARTICLE

Cite as: S. Doron *et al.*, *Science*
10.1126/science.aar4120 (2018).

Systematic discovery of antiphage defense systems in the microbial pangenome

Shany Doron,* Sarah Melamed,* Gal Ofir, Azita Leavitt, Anna Lopatina, Mai Keren, Gil Amitai, Rotem Sorek†