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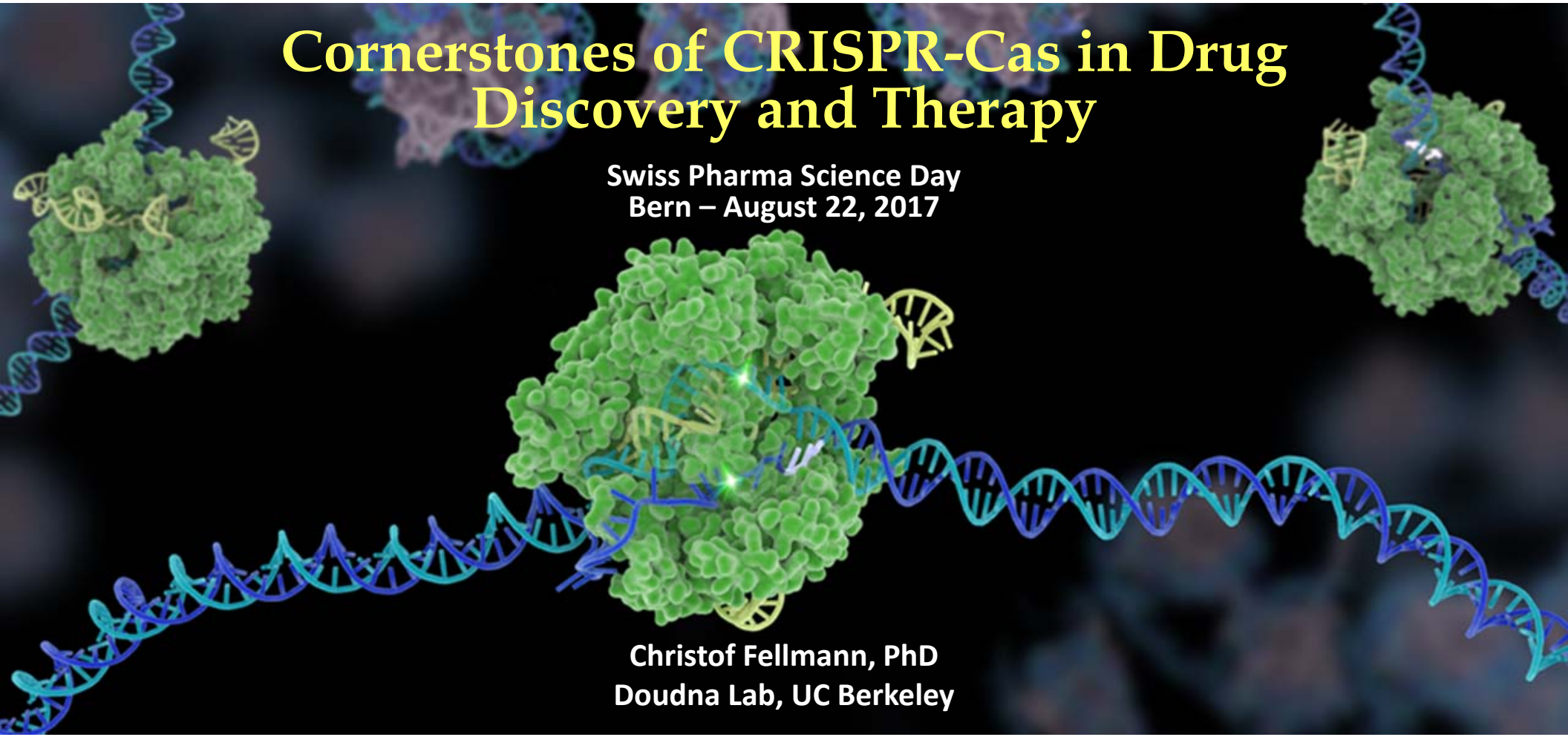
NIGMS

Cornerstones of CRISPR-Cas in Drug Discovery and Therapy

Swiss Pharma Science Day
Bern – August 22, 2017

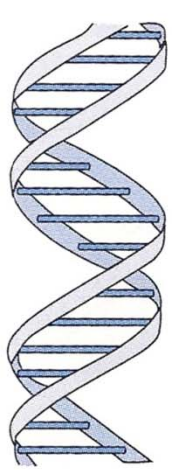


Christof Fellmann, PhD
Doudna Lab, UC Berkeley

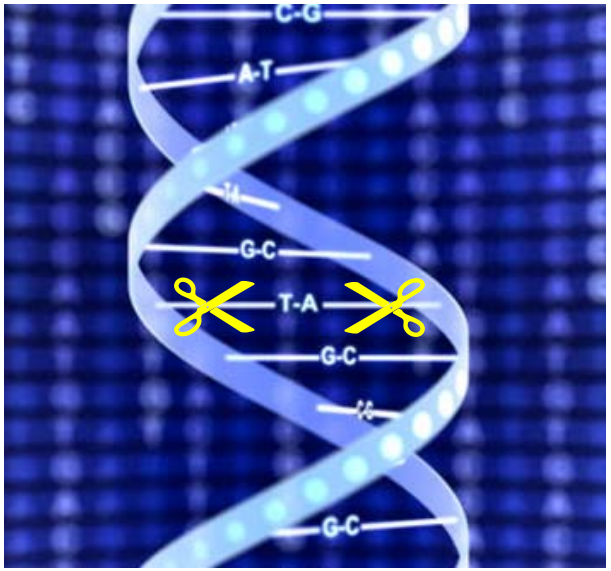
From Immune System to Precision Medicine

- Leveraging bacterial immunity for biomedicine
- RNA-guided precision genome engineering
- CRISPR-Cas for drug development
- CRISPR-Cas as *ex-vivo* and *in-vivo* therapeutic

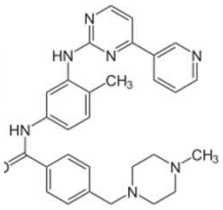
CRISPR-Cas – A revolutionary genome engineering technology



DNA



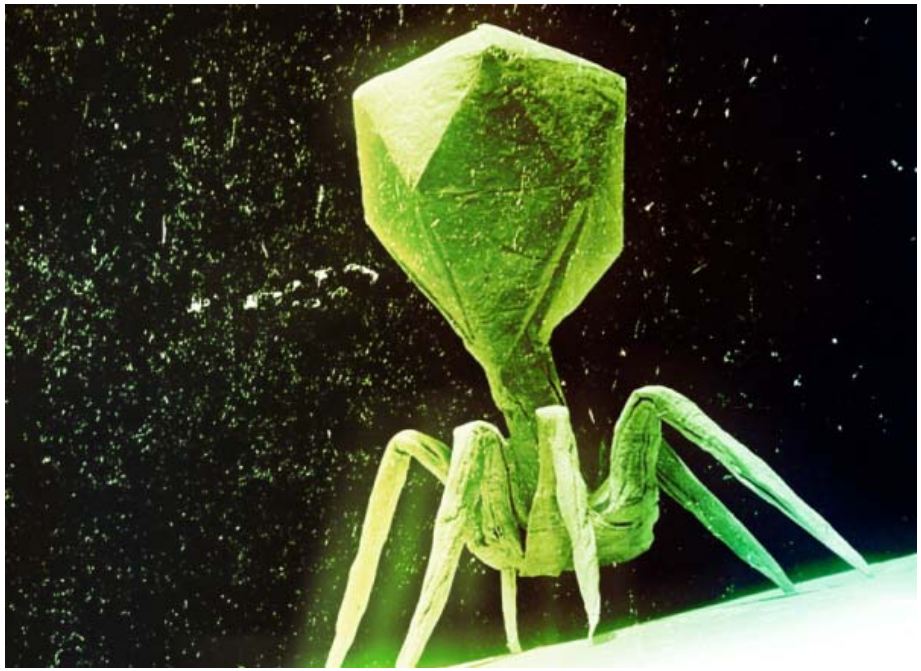
Protein

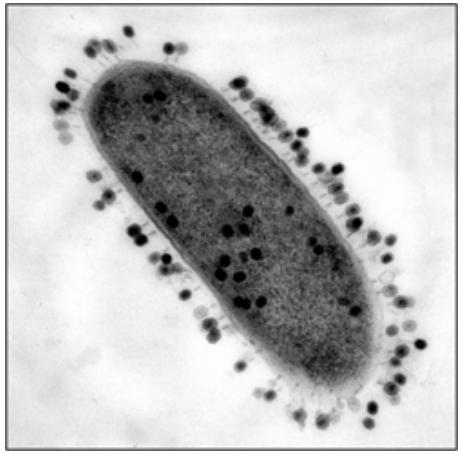


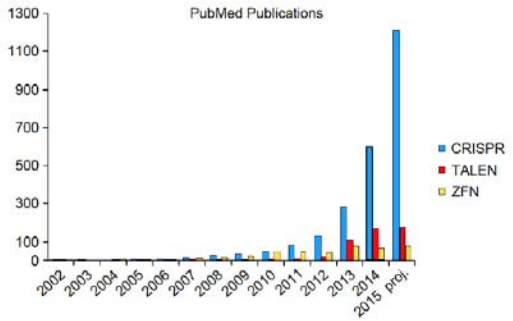
The central dogma of molecular biology

- Unidirectional flow of genetic information
- Medical approaches interact at the protein level
- What if we could rewrite the genetic code?

Leveraging bacterial immunity for biomedicine





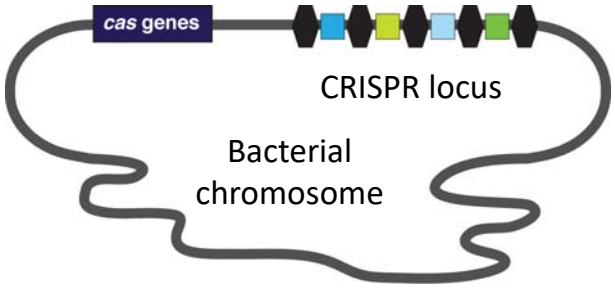


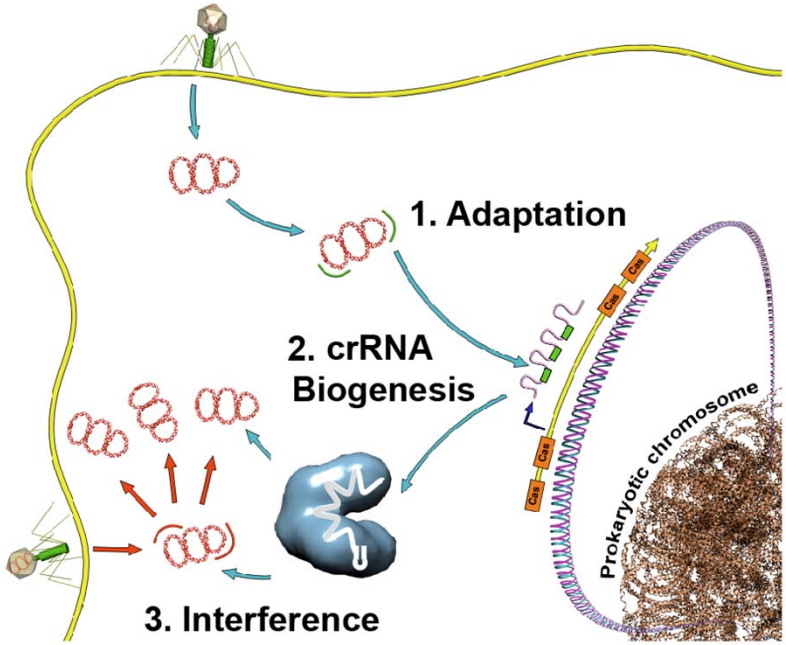
Curiosity about how bacteria fight the flu

- RNA-guided immune systems
- RNAi, ZFN, TALEN
- CRISPR-Cas9

http://msms.ehe.osu.edu
http://healtherandhappier.blogspot.com
Origamiwolf: https://flic.kr/p/oDCK1
Rodolphe Barrangou

CRISPRs – Acquired immune systems in bacteria



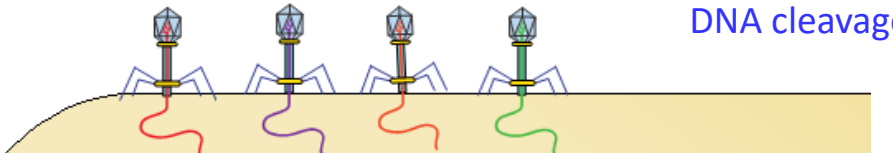


Clustered Regularly Interspaced Short Palindromic Repeats

- Extrachromosomal and phage-associated origins of spacers
- Defense against bacteriophages
- Streptococcus pyogenes (SpyCas9)

Ishino *et al.*, 1987
Bolotin *et al.*, 2005
Mojica *et al.*, 2005
Pourcel *et al.*, 2005

Bacterial CRISPR-Cas adaptive immunity



How does RNA-guided DNA cleavage work?

Nuñez *et al.*, Nature (2015a, 2015b), Nuñez *et al.*, Mol Cell (2016)

From Immune System to Precision Medicine

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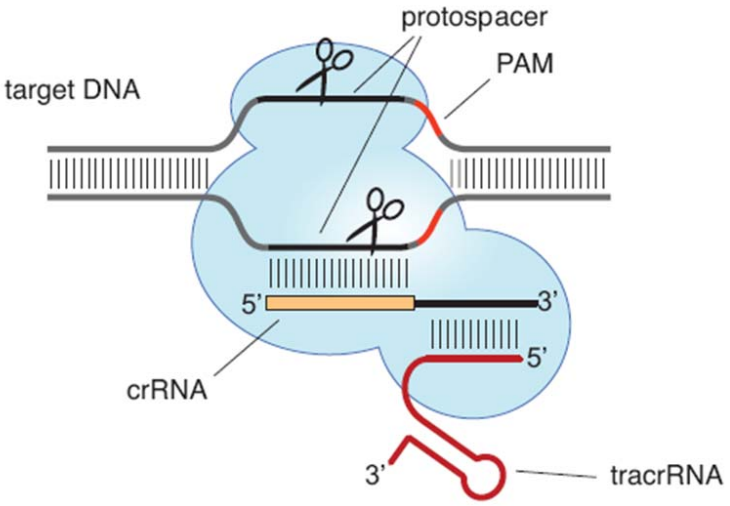
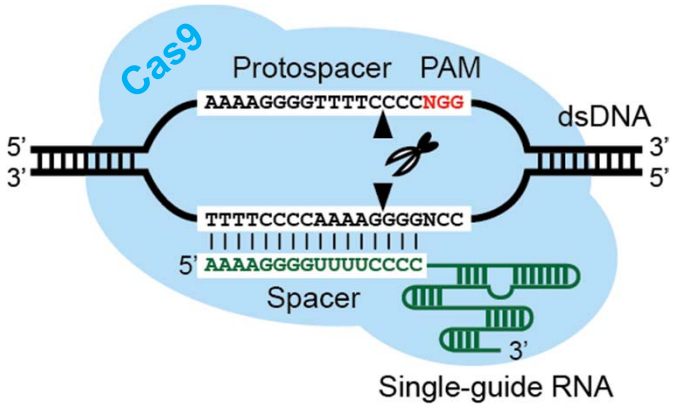
- CRISPR-Cas as *ex-vivo* and *in-vivo* therapeutic

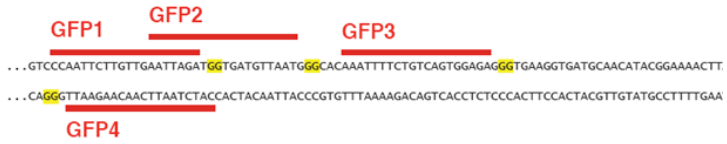
Programming Cas9 with single-guide RNAs (sgRNAs)

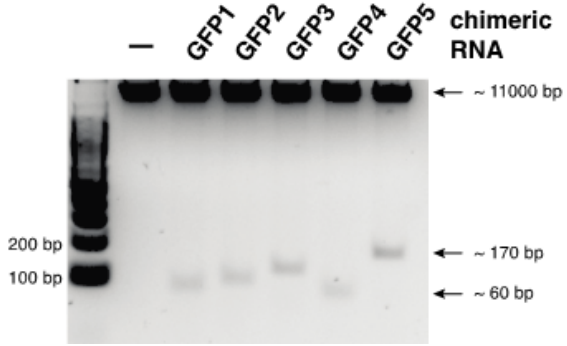
Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by sgRNA





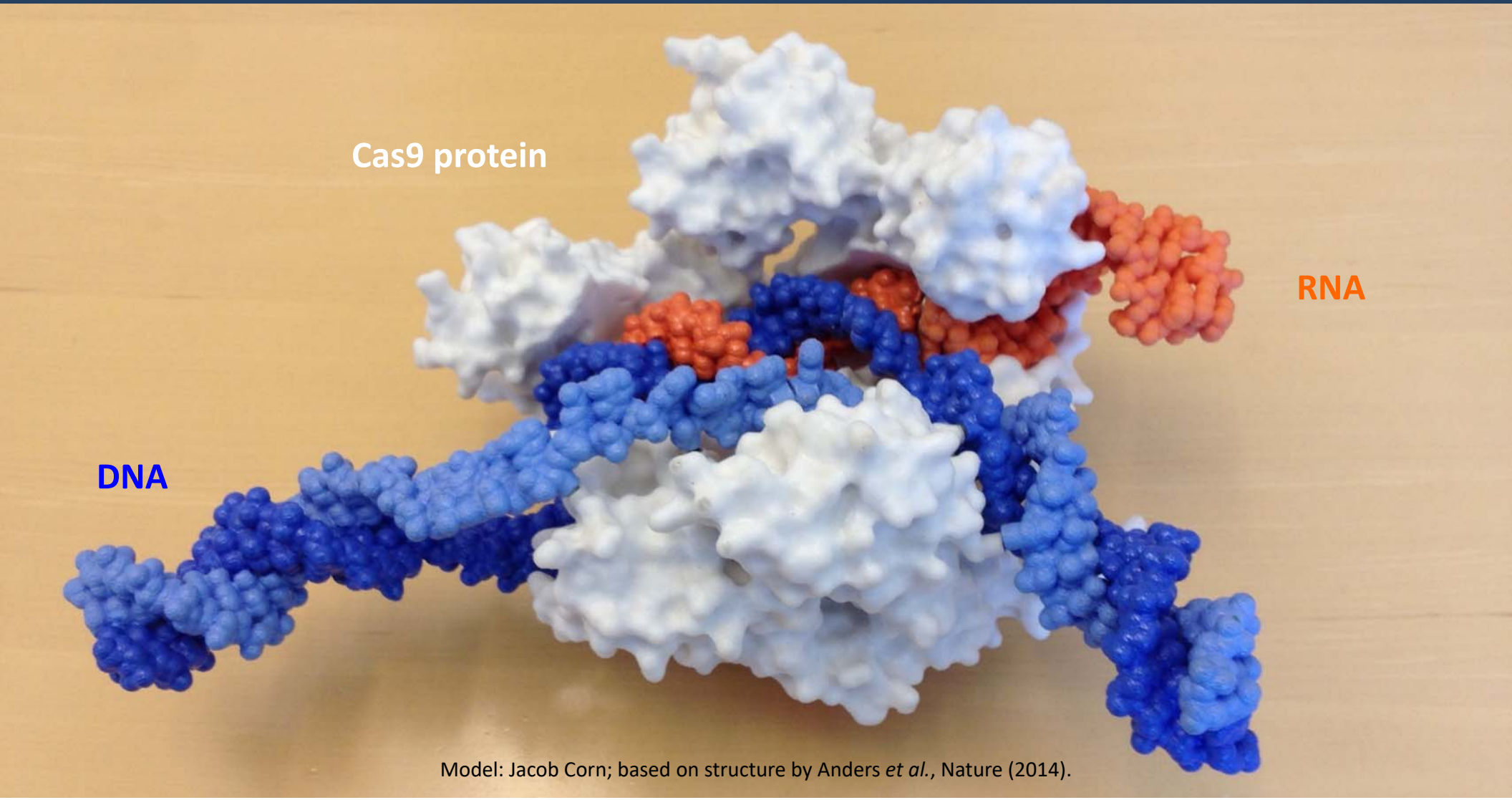


CRISPR-Cas9

- sgRNA guided
- PAM for target specificity
- Introduction of DNA DSBs

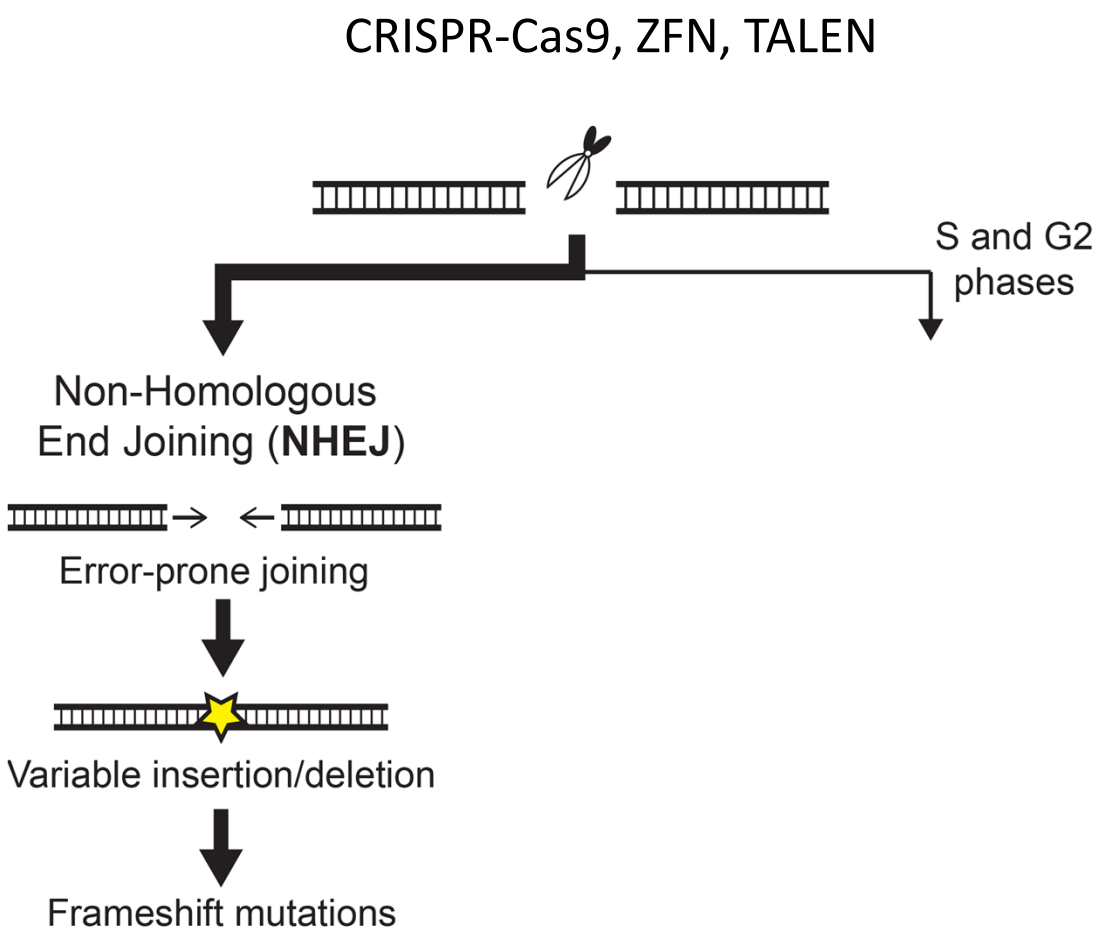
Jinek *et al.*, *Science* **337**, 816 (2012)

Cas9 and its guide RNA act like a molecular scalpel to cut DNA



Model: Jacob Corn; based on structure by Anders *et al.*, Nature (2014).

DNA DSB repair pathways – NHEJ, HDR (genome editing)



From Immune System to Precision Medicine

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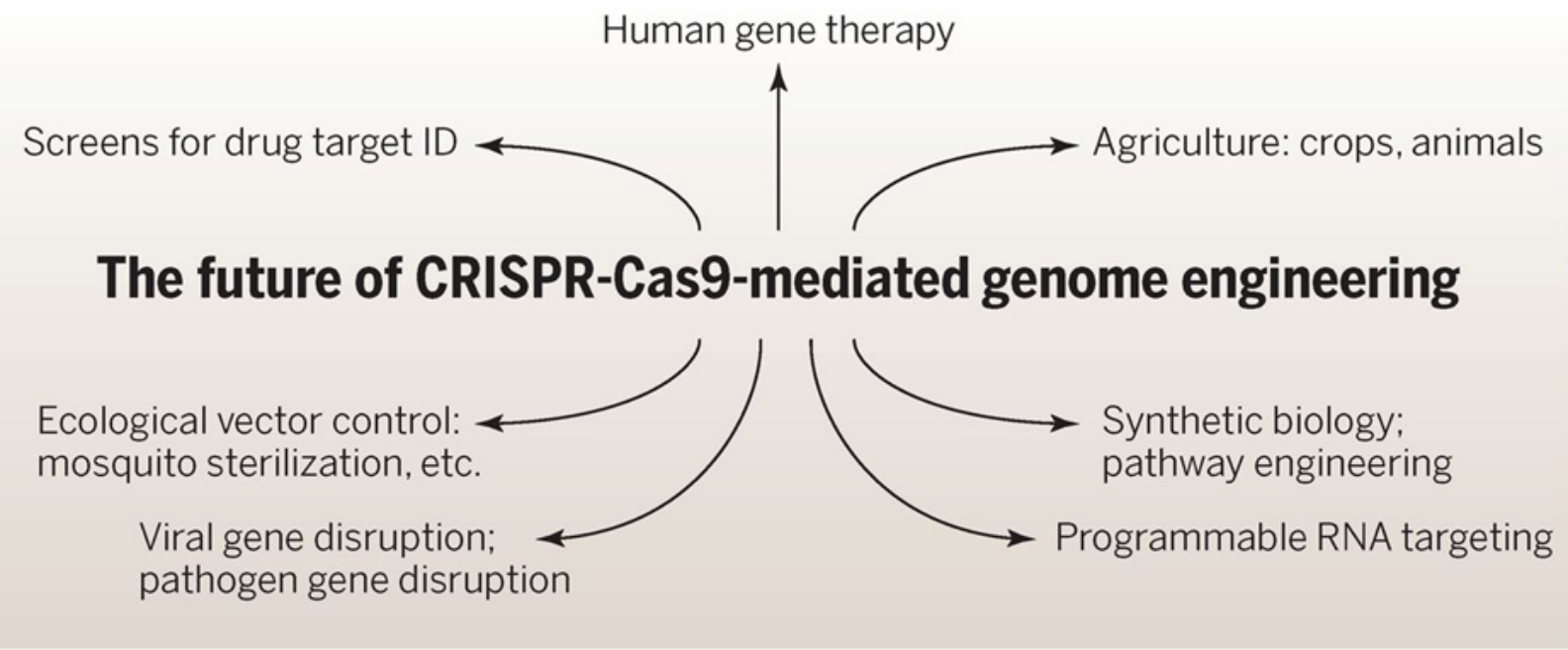
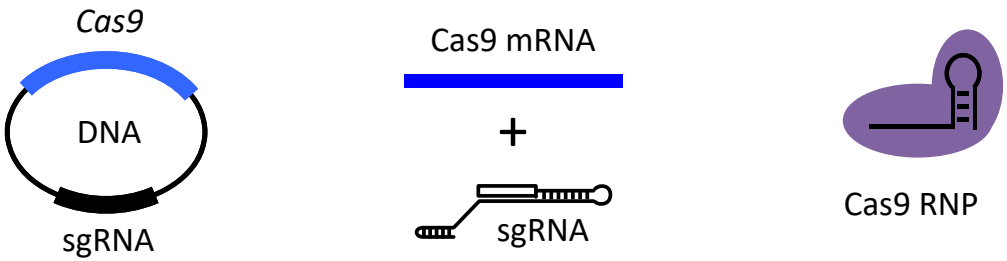
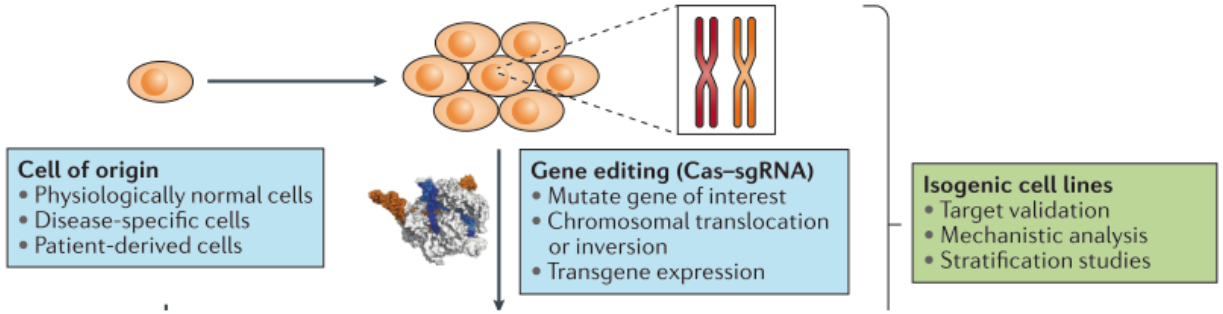
CRISPR-Cas genome engineering





Doudna and Charpentier, *Science* (2014)

CRISPR-Cas as a tool for drug discovery – in cell culture



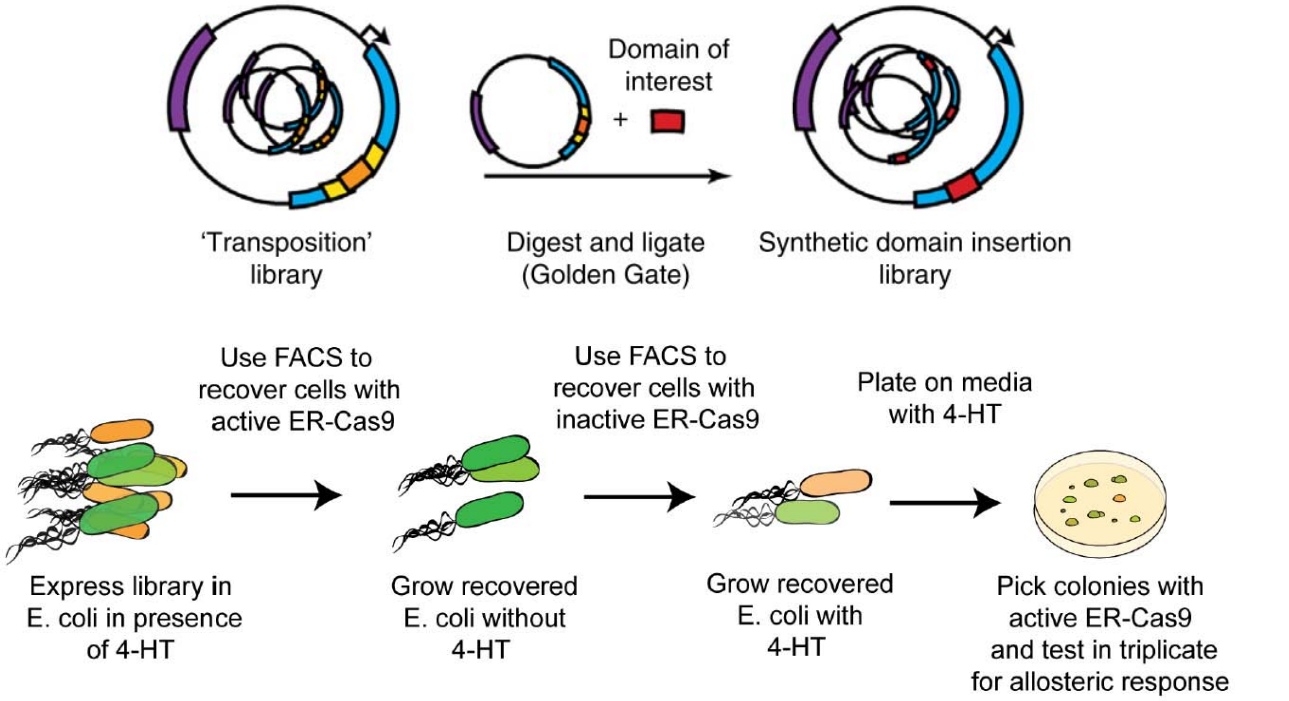
- CRISPR-Cas9
- CRISPRi (interference)
- CRISPRa (activation)

NRDD (2017)

Generation of a switch-like (inducible) Cas9

Allosterically regulated Cas9

- Insertion of the estrogen receptor alpha ligand binding domain (ER-LBD)
- dCas9 transposition library
- Assessment of large-scale library in *E. coli* (CRISPRi screen)

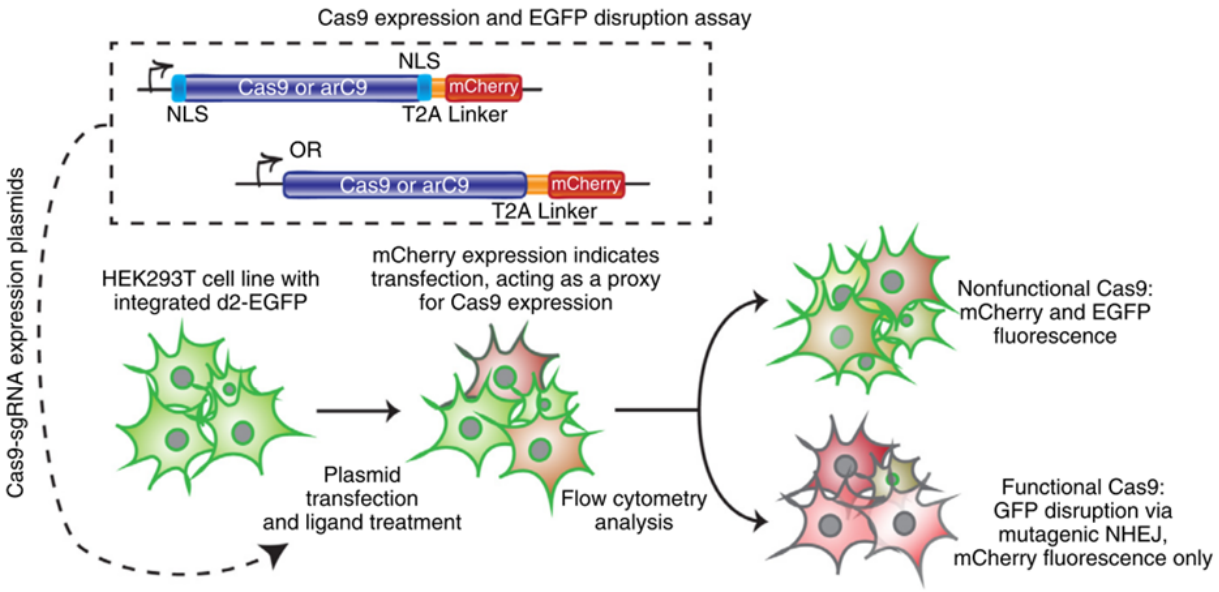


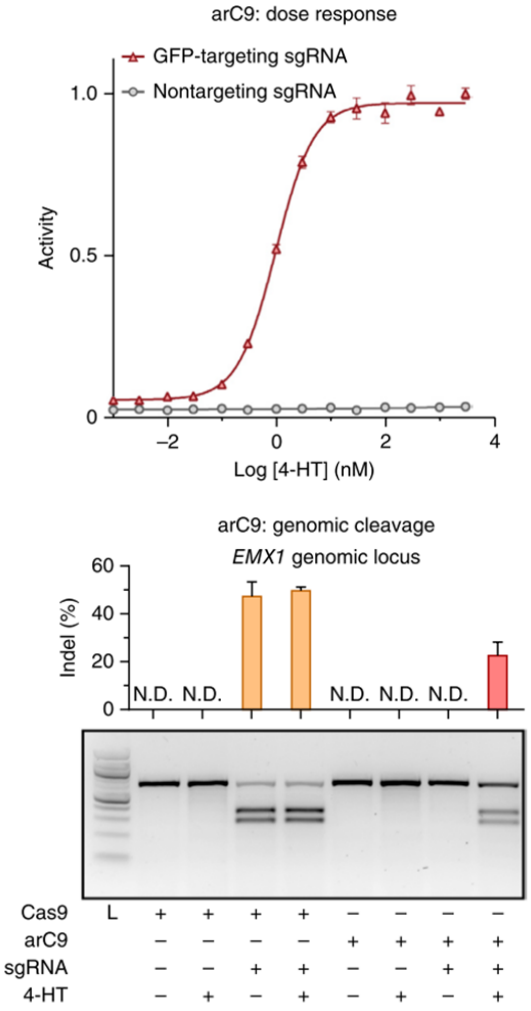
Oakes, *et al.*, Nat Biotech (2016)

Generation of a switch-like (inducible) Cas9

Allosterically regulated Cas9

- 4-OH Tamoxifen regulated (like Cre-ER system)
- Validation in mammalian cells



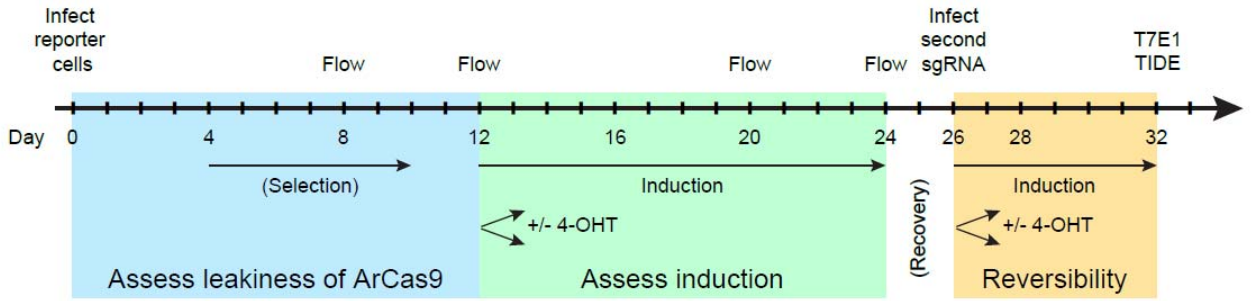


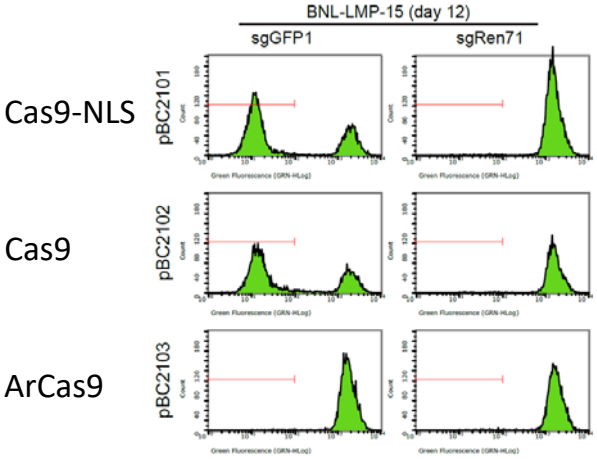
Oakes, *et al.*, Nat Biotech (2016)

Inducible Cas9 – ArCas9

Allosterically regulated Cas9

- Serially inducible and reversible
- GFP reporter cell line (BNL-LMP-15)





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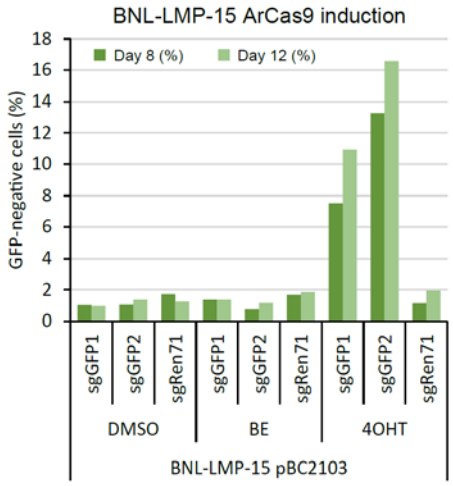
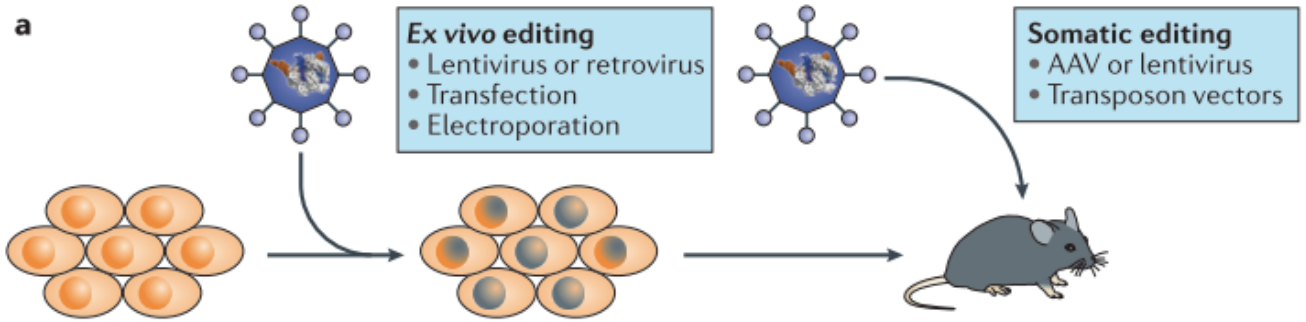


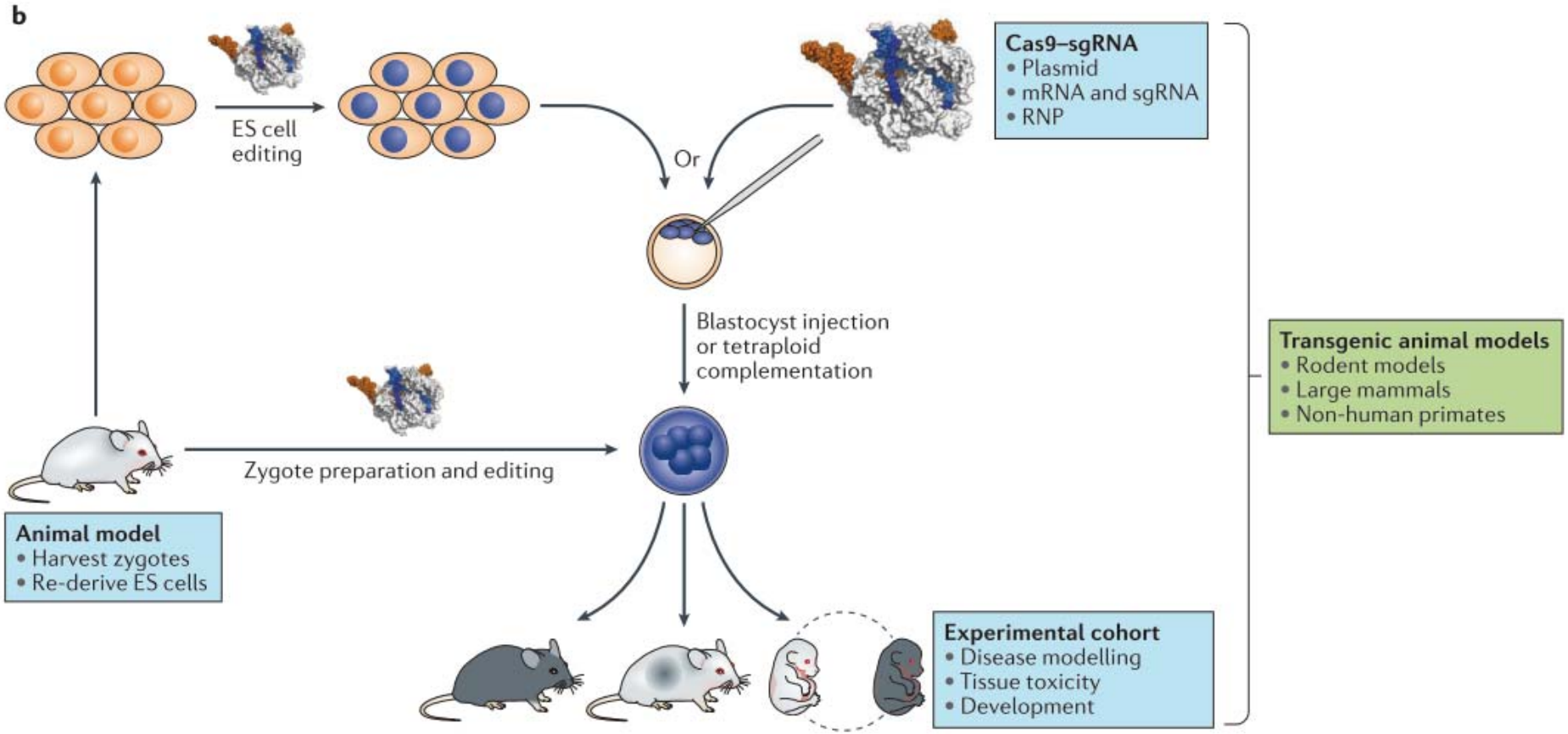
CRISPR-Cas as a tool for drug discovery – animal models

- CRISPR-Cas9
- CRISPRi (interference)
- CRISPRa (activation)



Fellmann *et al.*, NRDD (2017)

CRISPR-Cas as a tool for drug discovery – animal models



Fellmann *et al.*, NRDD (2017)

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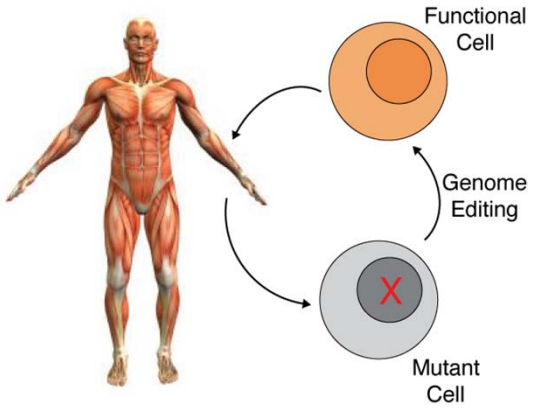
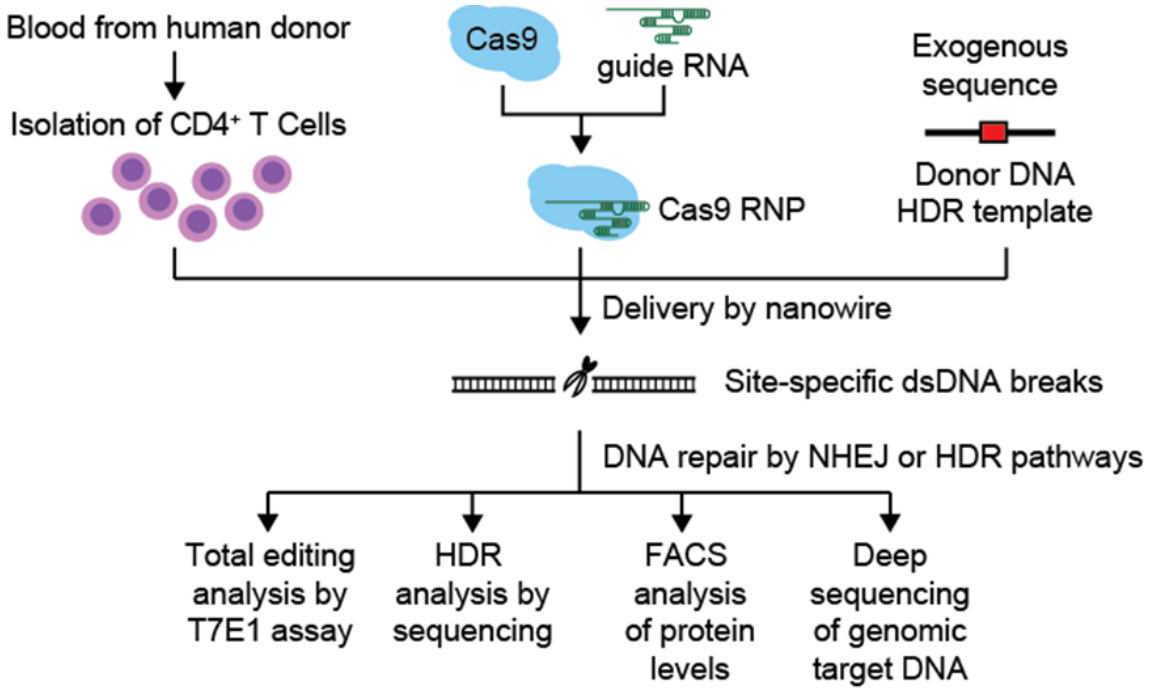
Ex-vivo genome editing – recoding primary human T cells



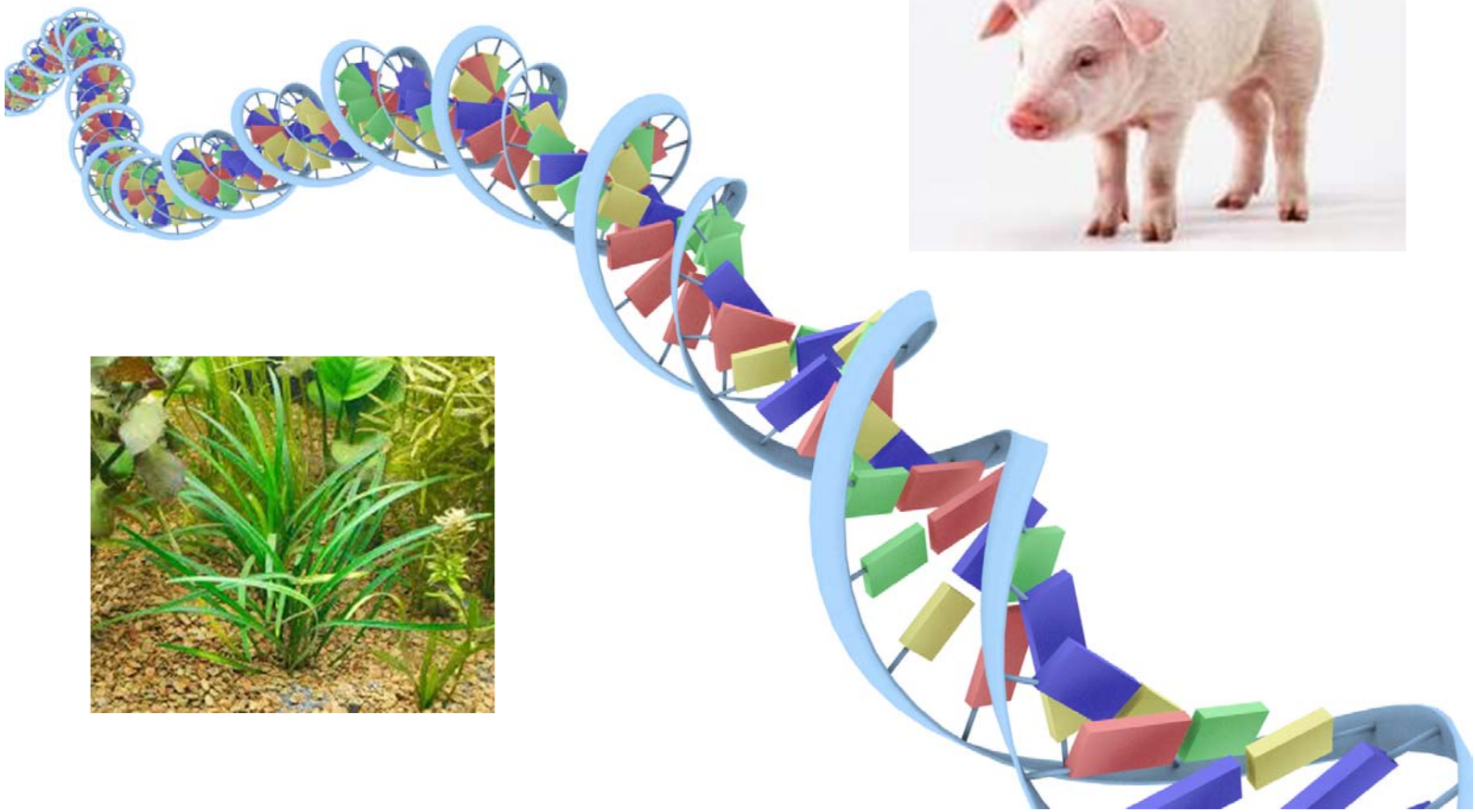
CXCR4, CCR5 editing for HIV treatment



Schumann, Lin *et al.*, *PNAS* (2015).
Shumann, Marson, Puck – UCSF
Martin – CHORI
Corn – Innovative Genomics Initiative

A prudent path forward for genome engineering

What should we do now that genomes can be edited easily?



A prudent path forward for genome engineering

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a hand-

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

A prudent path forward for genome engineering

GENE EDITING

In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar,[1,2]* Kexian Zhu,[1,3]* Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1]† Ru Xiao,[8] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[8] George M. Church,[4] Amy J. Wagers[1]‡

GENE EDITING

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13]*

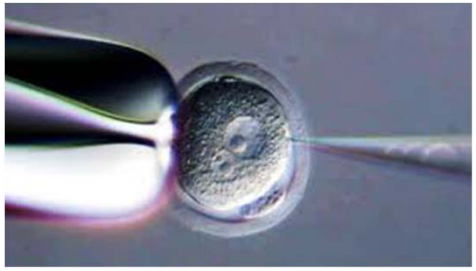
GENE EDITING

Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

Chengzu Long,[1,2,3]* Leonela Amoasii,[1,2,3]* Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3]†

A prudent path forward for genome engineering

What should we do now that genomes can be edited easily?



PERSPECTIVES

BIOTECHNOLOGY

A prudent path forward for genomic engineering and germline gene modification

A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed

By **David Baltimore,[1] Paul Berg,[2] Michael Botchan,[3,4] Dana Carroll,[5] R. Alta Charo,[6] George Church,[7] Jacob E. Corn,[4] George Q. Daley,[8,9] Jennifer A. Doudna,[4,10*] Marsha Fenner,[4] Henry T. Greely,[11] Martin Jinek,[12] G. Steven Martin,[13] Edward Penhoet,[14] Jennifer Puck,[15] Samuel H. Sternberg,[16] Jonathan S. Weissman[4,17], Keith R. Yamamoto,[4,18]**

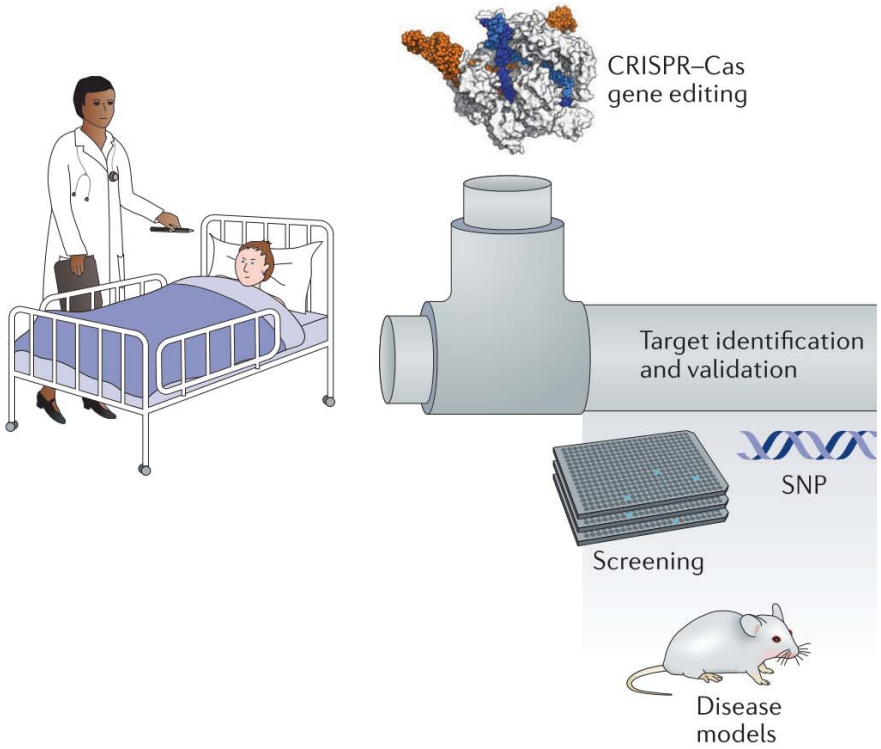
ture developments. The meeting identified immediate steps to take toward ensuring that the application of genome engineering technology is performed safely and ethically.

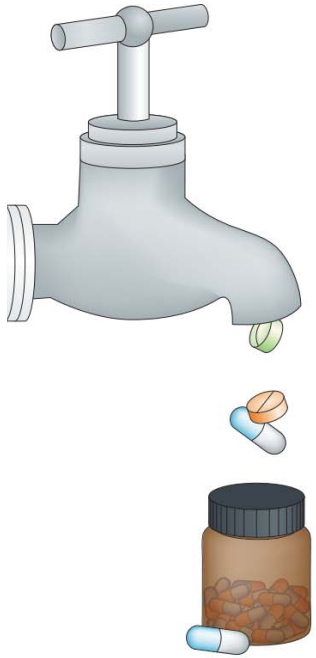
The promise of so-called "precision medicine" is propelled in part by synergies between two powerful technologies: DNA sequencing and genome engineering. Advances in DNA sequencing capabilities and genome-wide association studies have provided critical information about the ge-

CURRENT APPLICATIONS. The simplicity of the CRISPR-Cas9 system allows any researcher with knowledge of molecular biology to modify genomes, making feasible experiments that were previously difficult or impossible to conduct. For example, the CRISPR-Cas9 system enables introduction of DNA sequence changes that correct genetic defects in whole animals, such as replacing a mutated gene underlying liver-based metabolic disease in a mouse model (*3*). The tech-

Baltimore, *et al.*, Science (2015)

From microbial immune system to precision medicine





Accelerate drug discovery and therapy

- CRISPR-Cas editing, inhibition or activation (functional genomics)
- Disease models
- Cellular therapies

Fellmann *et al.*, NRDD (2017)

Acknowledgements

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D-Lab

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Thank you!