

OBJECTIVES

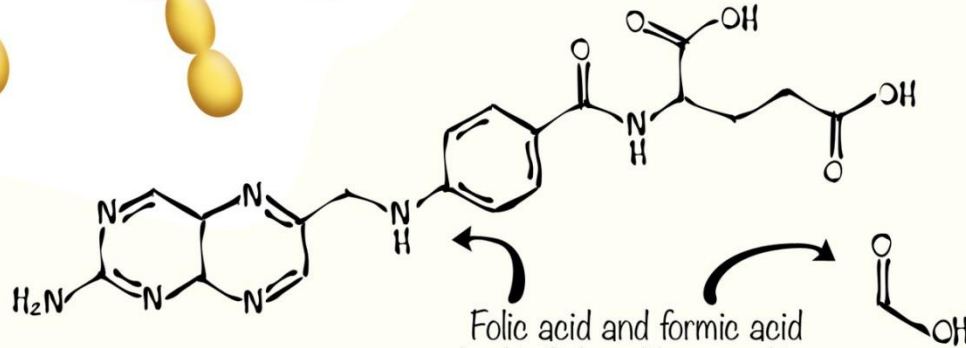


1. Describe the bacterial **CRISPR/Cas** locus and the sequences within
2. Relate the functioning of bacterial **CRISPR/Cas** systems to **acquired immunity**
3. Describe how **CRISPR/Cas9** cuts DNA
4. Explain how **CRISPR/Cas9** is used in **genome editing**
5. Provide **examples of CRISPR/Cas9 genome editing**

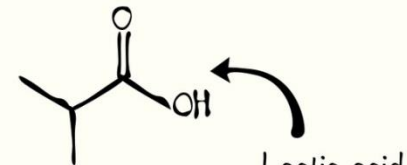


Streptococcus thermophilus

a non-pathogenic bacterium used in the production of fermented dairy products



Folic acid and formic acid produced by *S. thermophilus* are used by *Lactobacillus bulgaricus* for purine synthesis. The two species have a synergistic relationship that is exploited by yogurt and cheese producers.

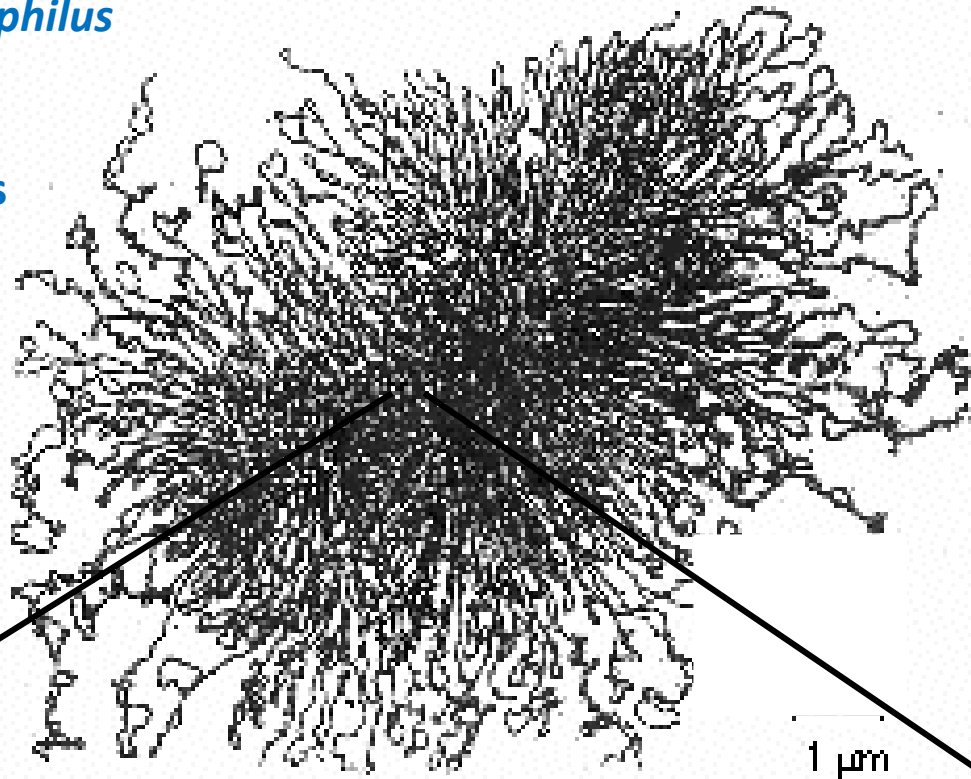


Lactic acid is a product of lactose fermentation by *S. thermophilus* in yogurt and cheese, which makes it possible for many lactose-intolerant people to consume these dairy products.

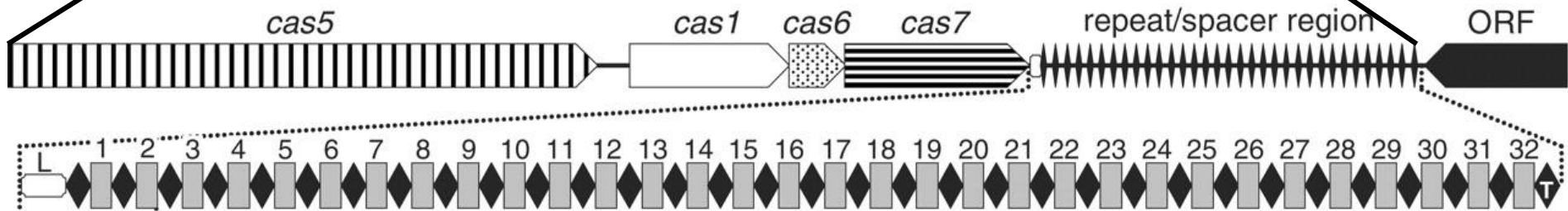
While most *Streptococcus* species are pathogenic, *S. thermophilus* is believed to have diverged about 3000 years ago, and adapted to dairy fermentation.

Streptococcus thermophilus
chromosome

~1.8 million base pairs
~1900 genes



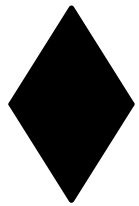
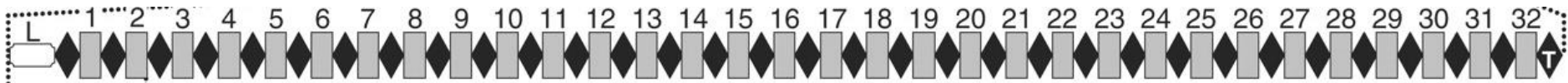
CRISPR/Cas Locus



Source: Barrangou *et al.* (2007) *Science*

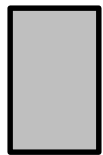
CRISPR LOCUS

(Clustered Regularly Interspersed Short Palindromic Repeats)



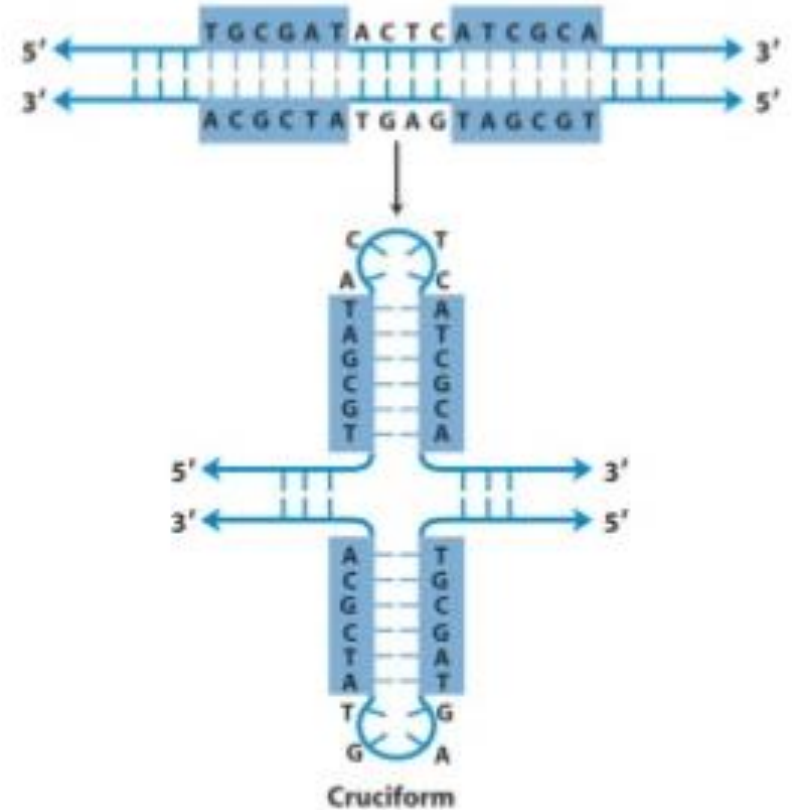
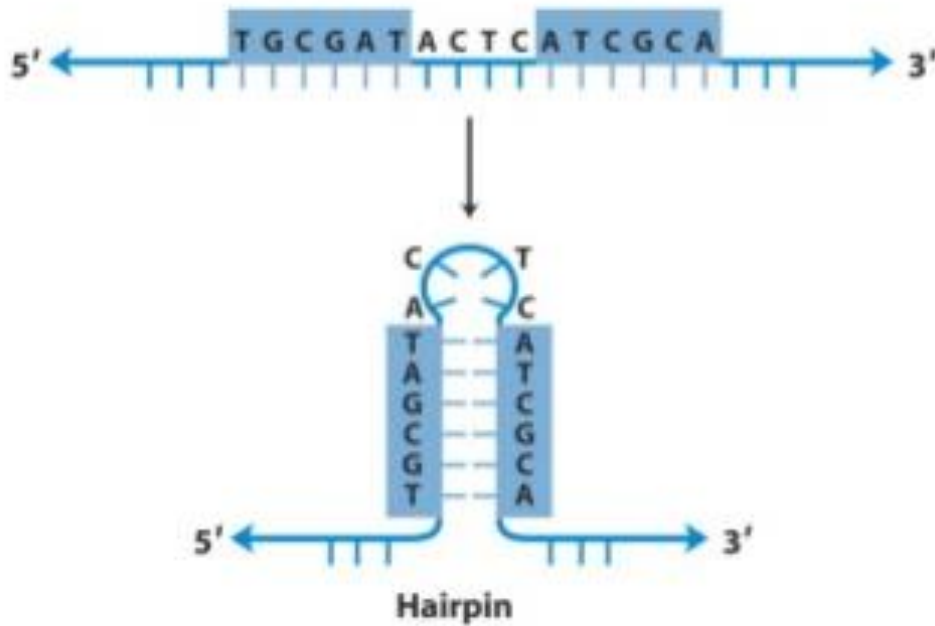
**= PALINDROMIC - Was it a car or a cat I saw?
REPEATS Madam, I'm Adam.**

A man, a plan, a canal, Panama!



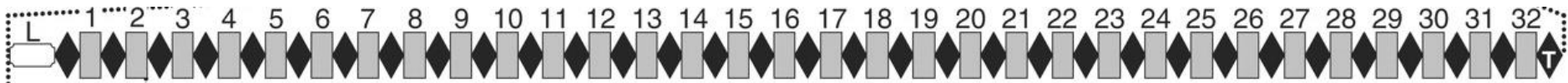
= SPACERS

PALINDROMIC SEQUENCES IN DNA CAN FOLD INTO DIFFERENT STRUCTURES



CRISPR LOCUS

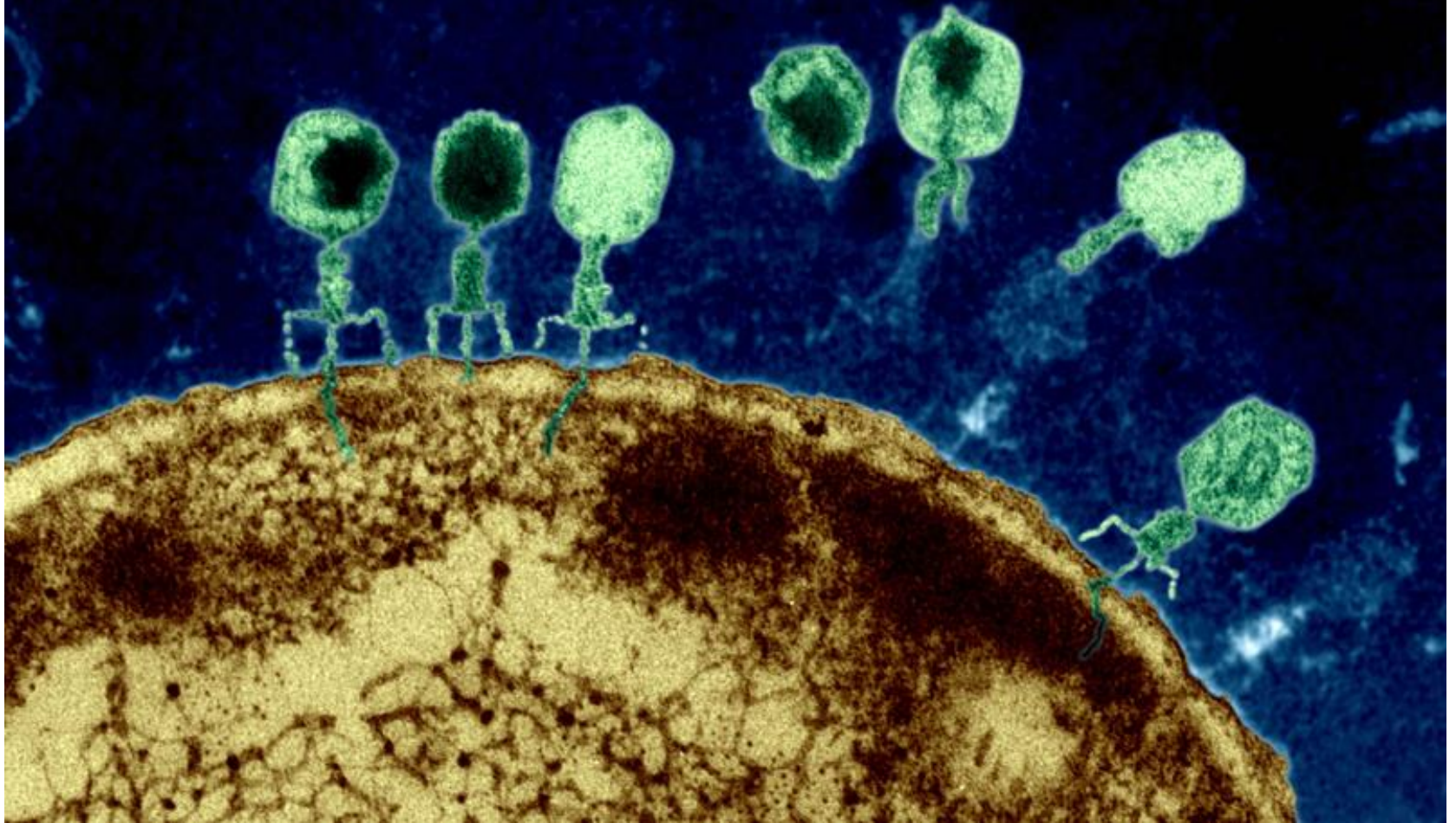
(Clustered Regularly Interspersed Short Palindromic Repeats)



 = PALINDROMIC REPEATS

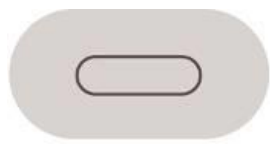
 = SPACERS

Bacteriophage (viruses that infect bacteria)

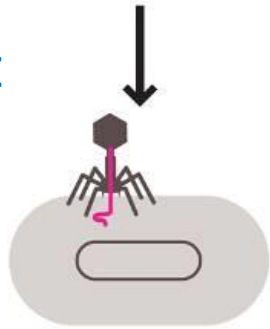


Source: Environmental Health Perspectives

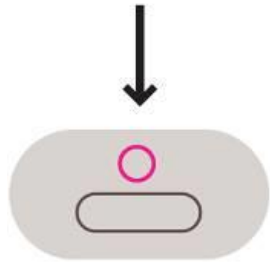
**BACTERIOPHAGE LIFE CYCLE
(LYTIC)**



BACTERIUM



injection of bacteriophage
DNA into bacterium

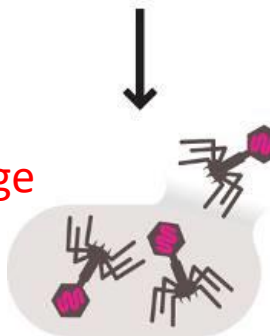


lytic growth



expression of bacteriophage
genes – synthesis of bacteriophage
proteins and replication bacteriophage DNA

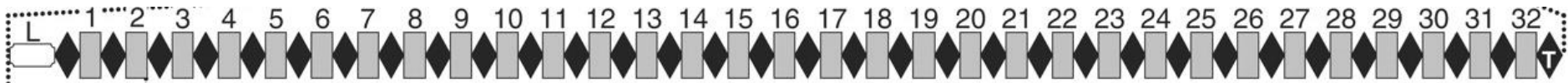
assembly of new bacteriophage
and lysis of infected cell



Source: *Molecular Biology, Craig et al.*

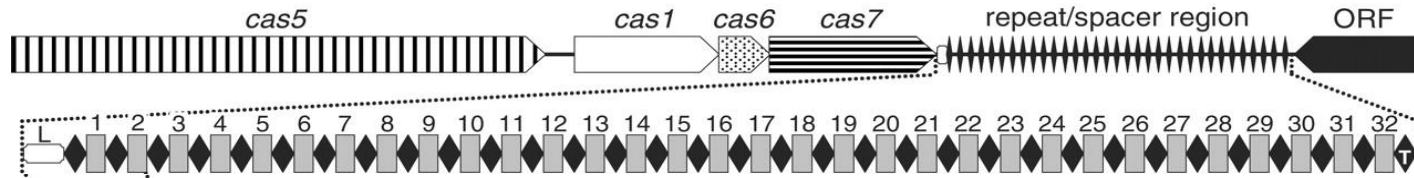
CRISPR LOCUS

(Clustered Regularly Interspersed Short Palindromic Repeats)



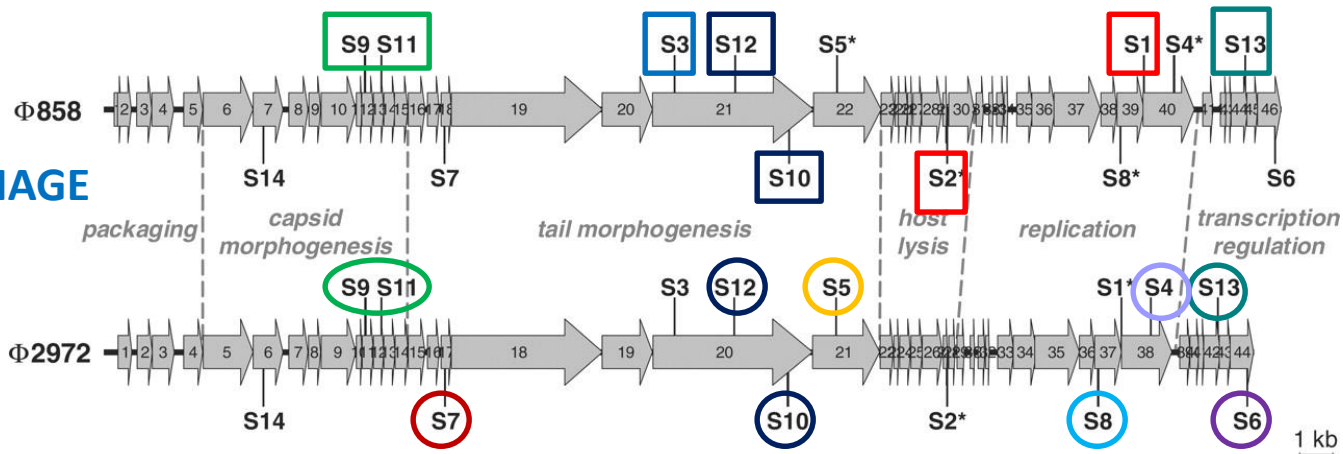
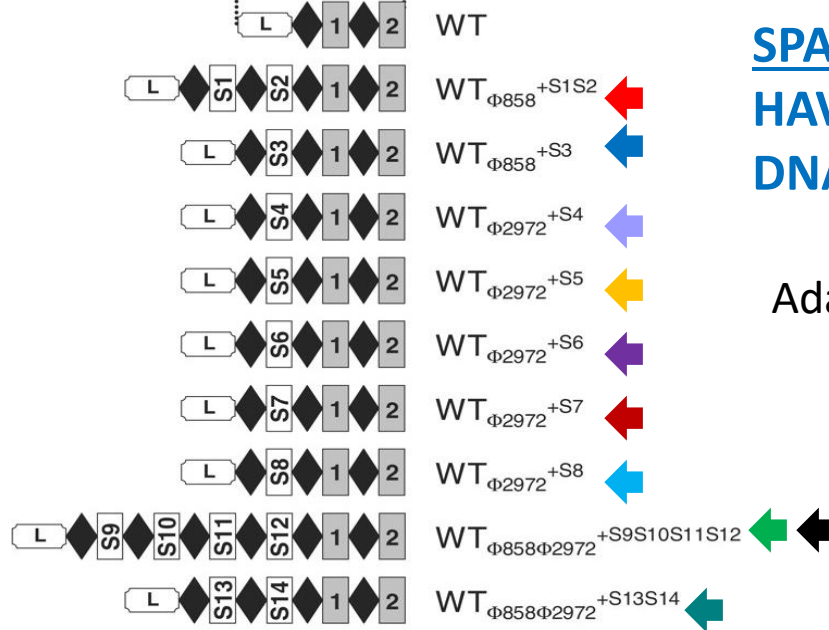
 = PALINDROMIC REPEATS

 = SPACERS



SPACER SEQUENCES IN CRISPR LOCUS HAVE HOMOLGY TO BACTERIOPHAGE DNA

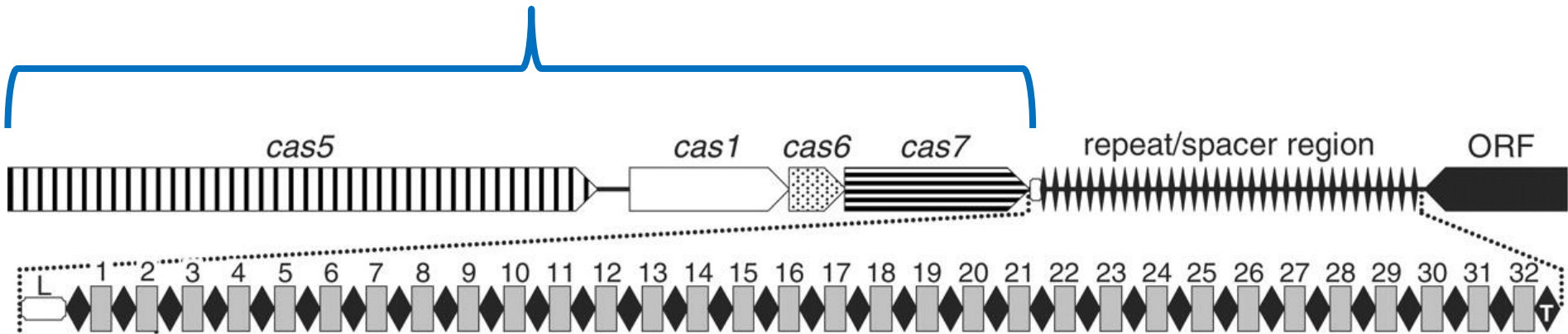
Adapted from Barrangou *et al.* (2007) *Science*



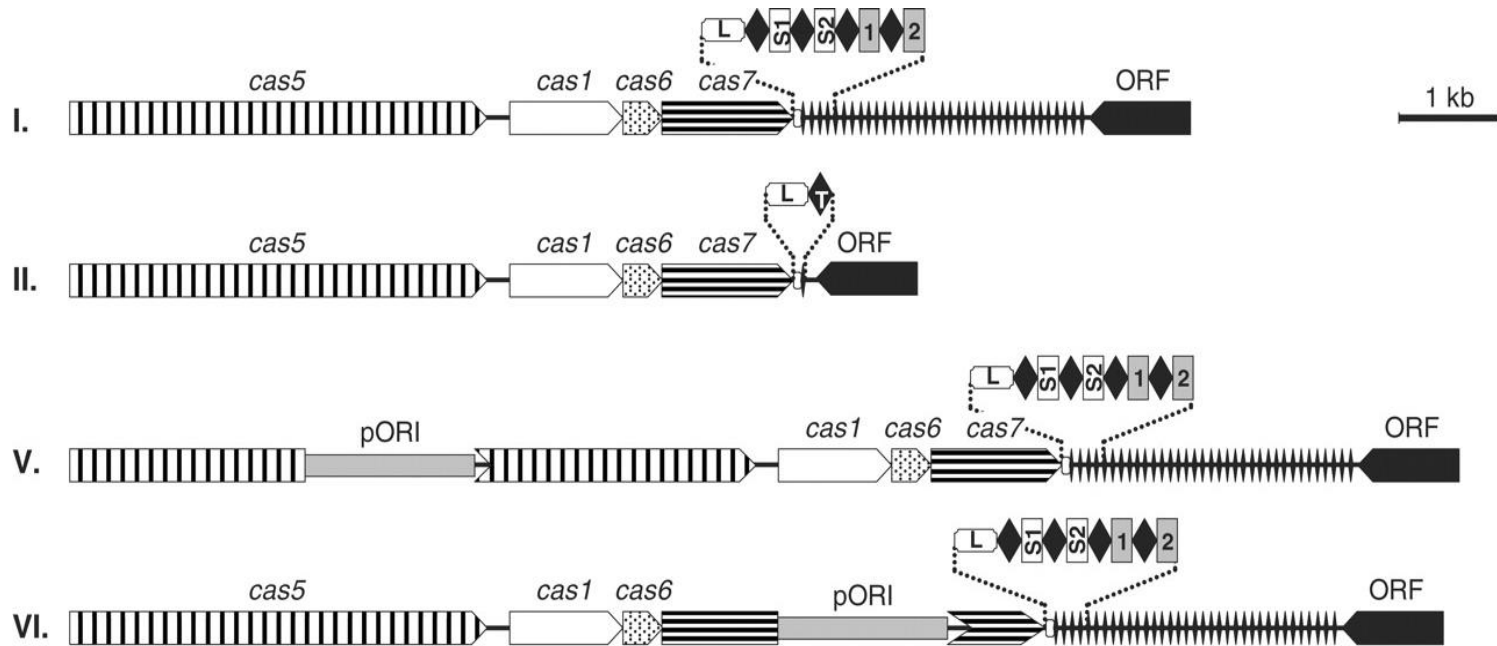
**BACTERIOPHAGE
GENES**

1 kb

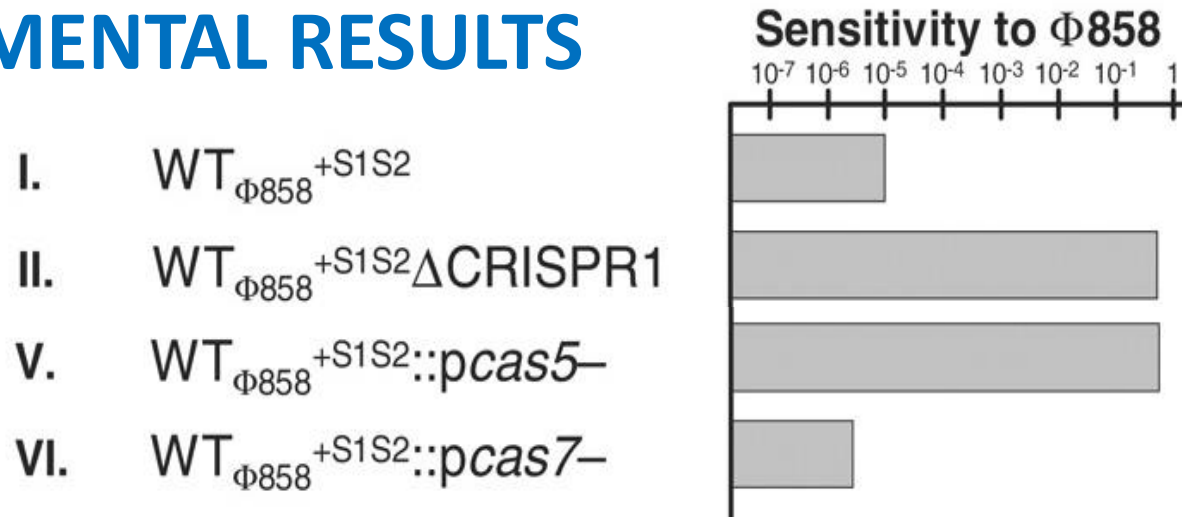
CRISPR/Cas (CRISPR Associated Loci)



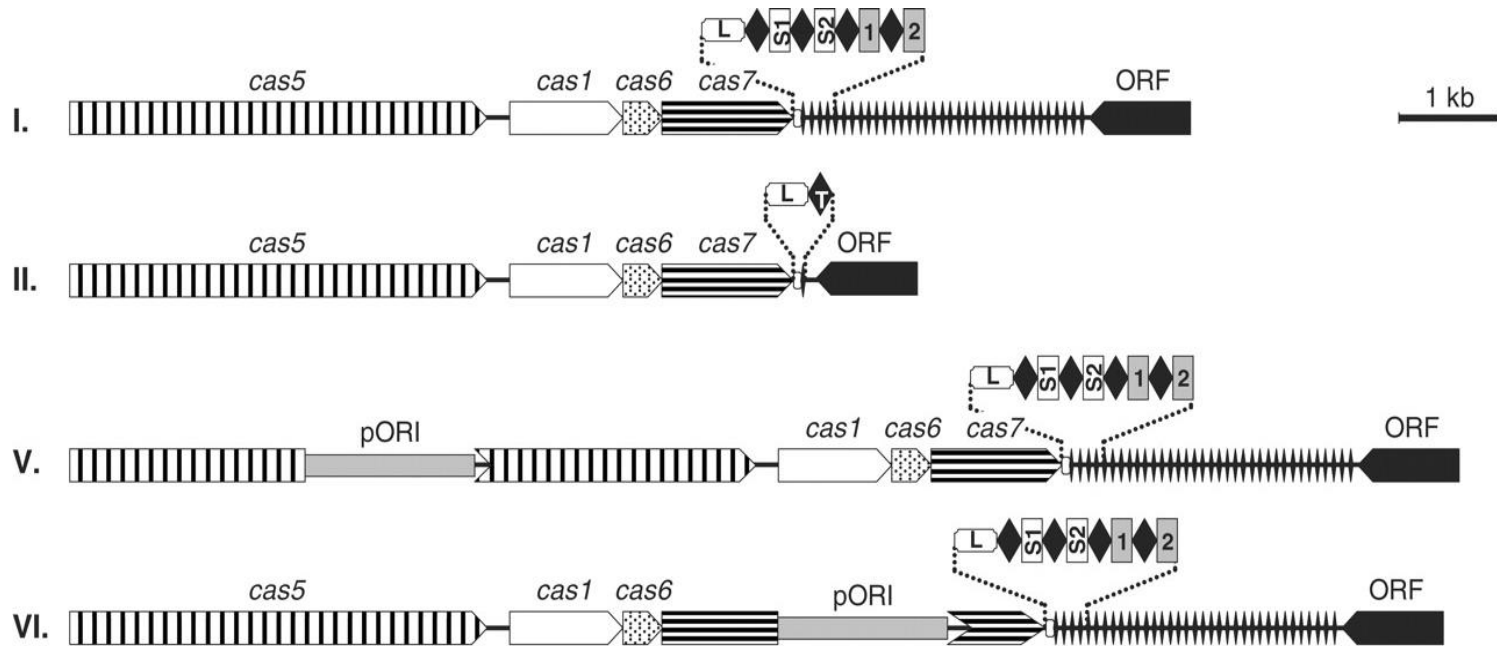
Cas GENES ENCODE PROTEINS AND RNA
INVOLVED IN **CRISPR** FUNCTION



EXPERIMENTAL RESULTS

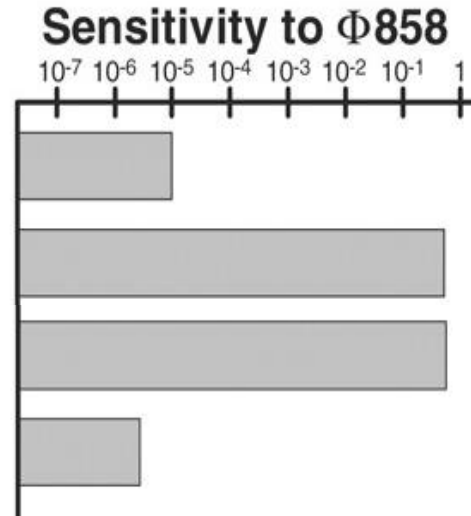


Adapted from Barrangou *et al.* (2007) *Science*



CRISPR LOCUS AND GENES PROVIDE PROTECTION AGAINST VIRAL INFECTION!!!!

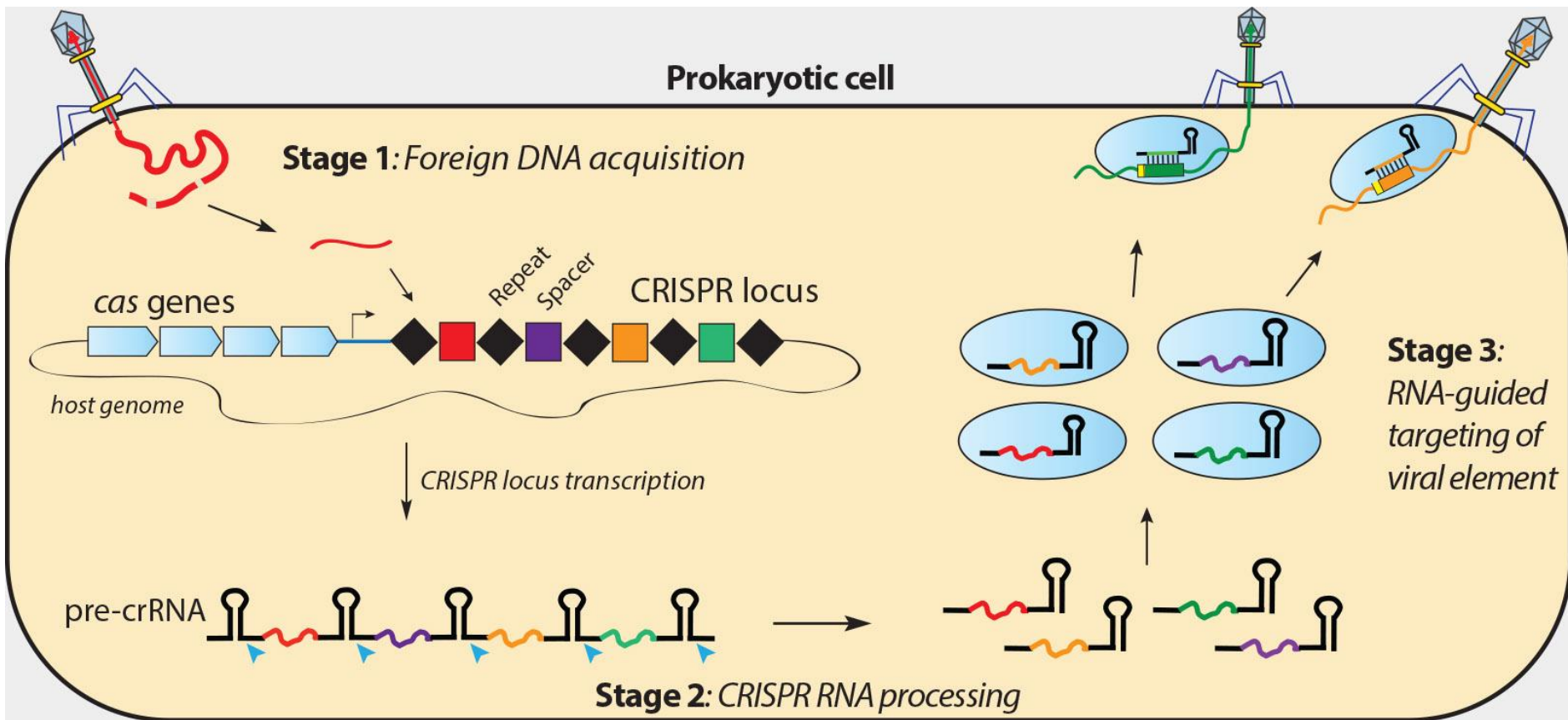
- I. $WT_{\Phi 858}^{+S1S2}$
- II. $WT_{\Phi 858}^{+S1S2\Delta CRISPR1}$
- V. $WT_{\Phi 858}^{+S1S2::pcas5-}$
- VI. $WT_{\Phi 858}^{+S1S2::pcas7-}$



Adapted from Barrangou *et al.* (2007) *Science*

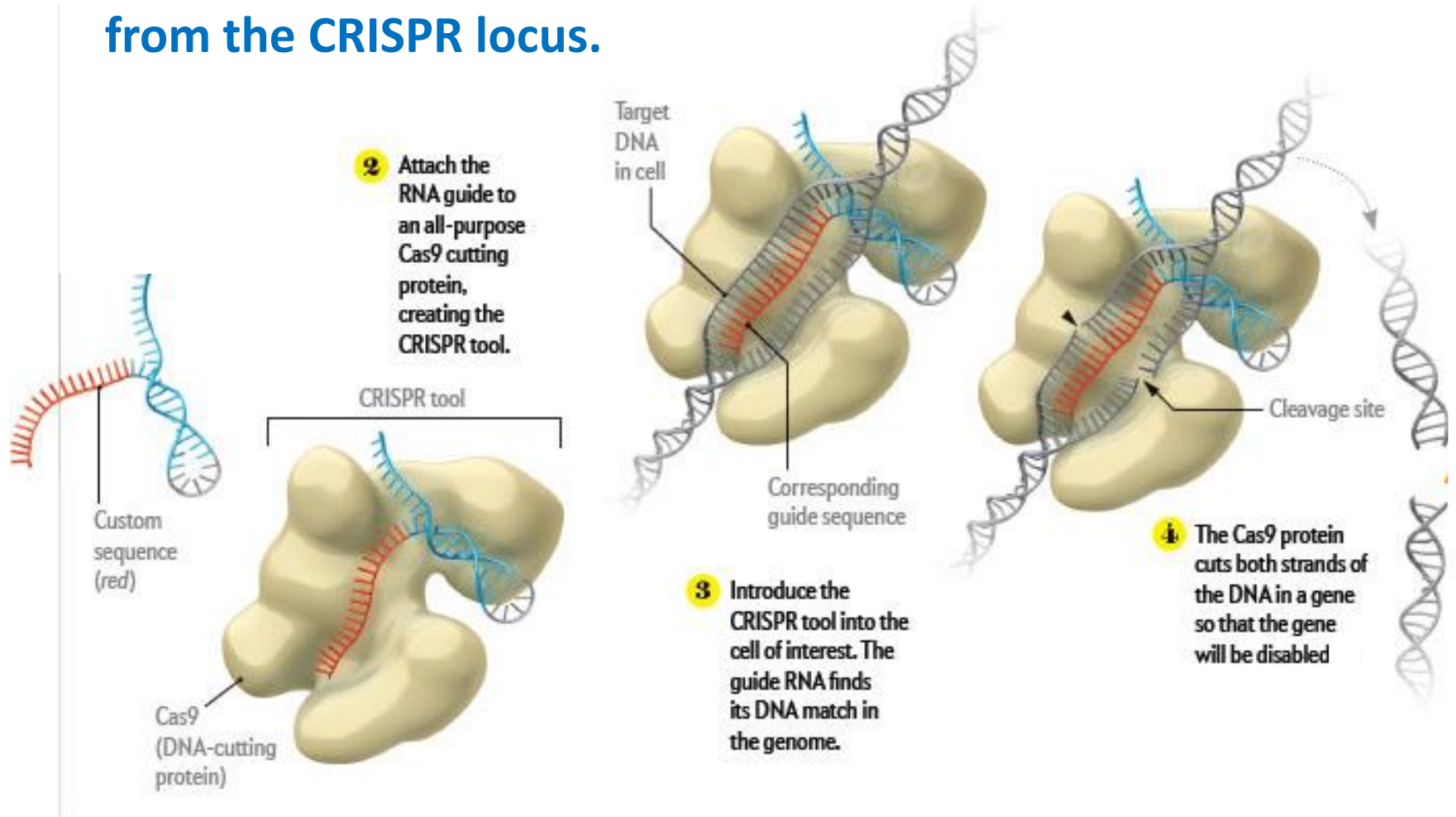
STAGES OF CRISPR/Cas9 FUNCTION

1. Acquisition of Foreign DNA
2. CRISPR RNA Processing
3. RNA-Guided Targeting of Foreign DNA



Source: Doudna Lab Website

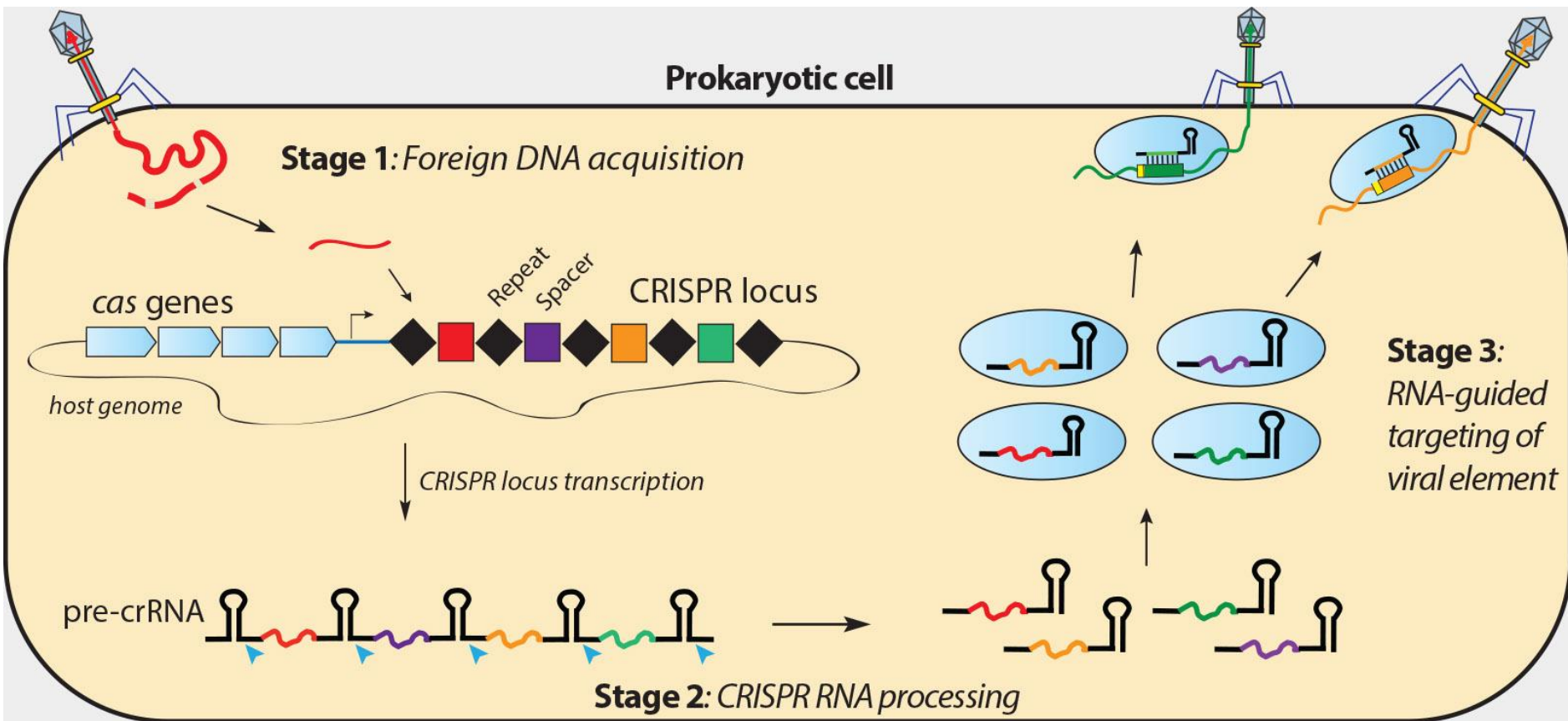
- Cas9 is a double-stranded endonuclease (an enzyme that cleaves both strands of DNA).
- Cas9's cleavage site is determined by RNA sequences derived from the CRISPR locus.



Source: Scientific American

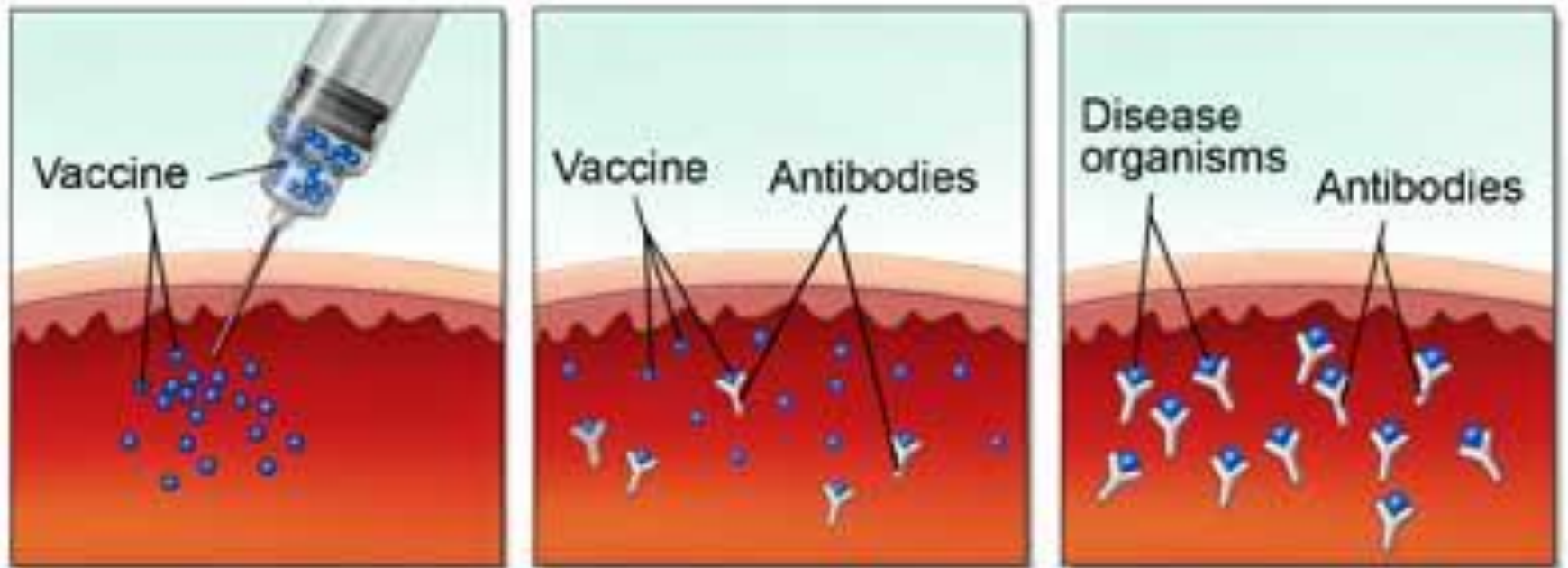
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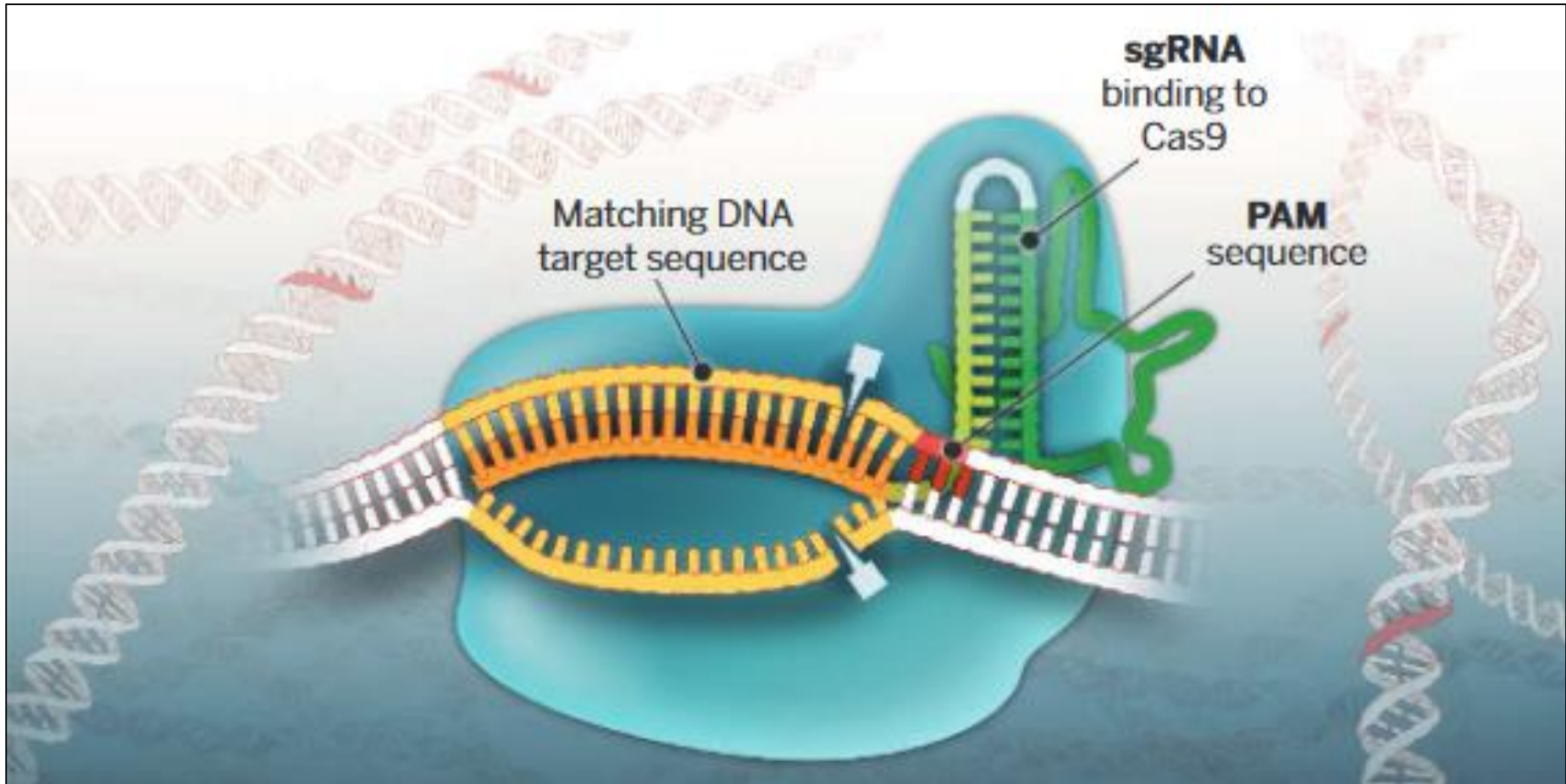


Source: Doudna Lab Website

Vaccine Immunity

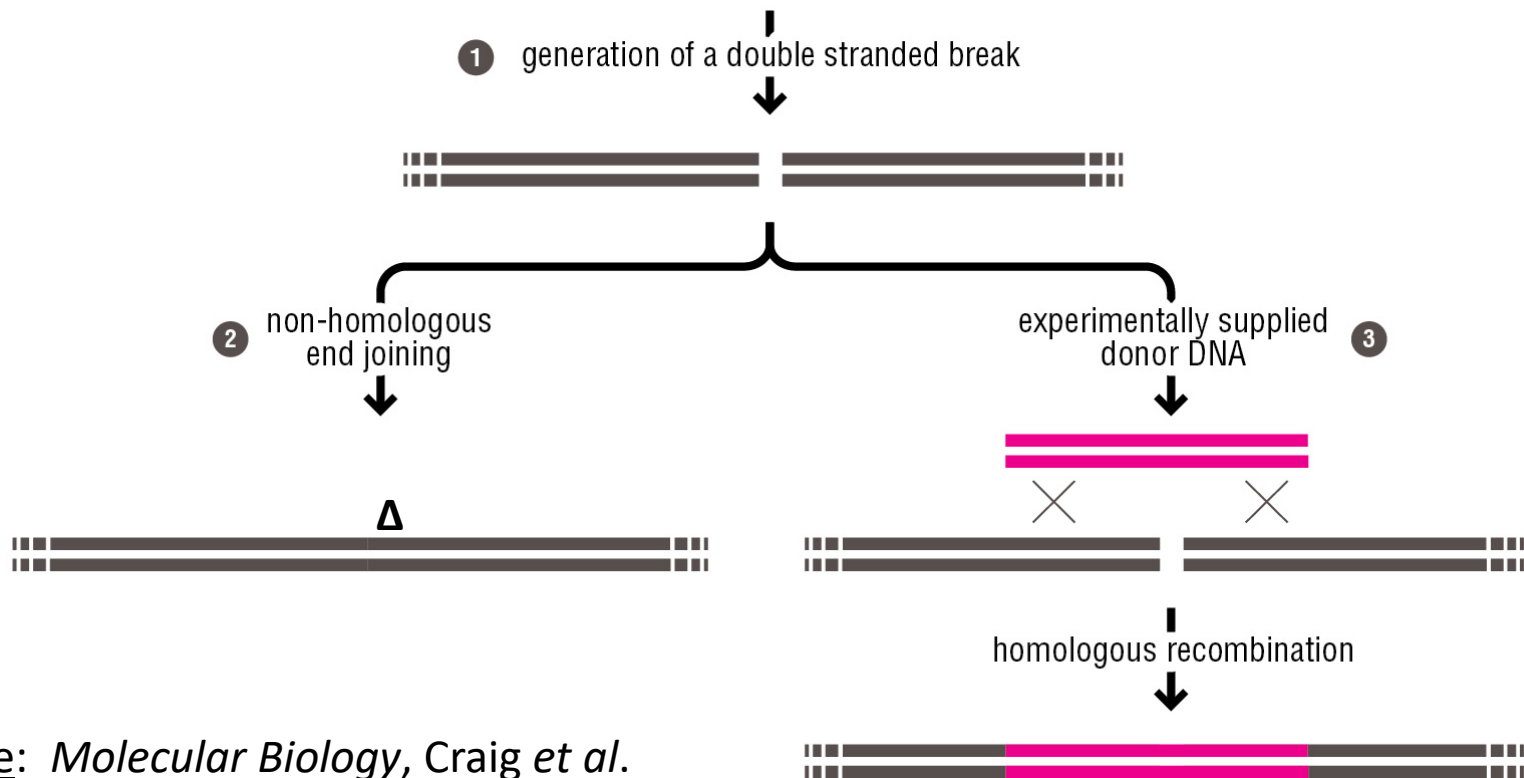
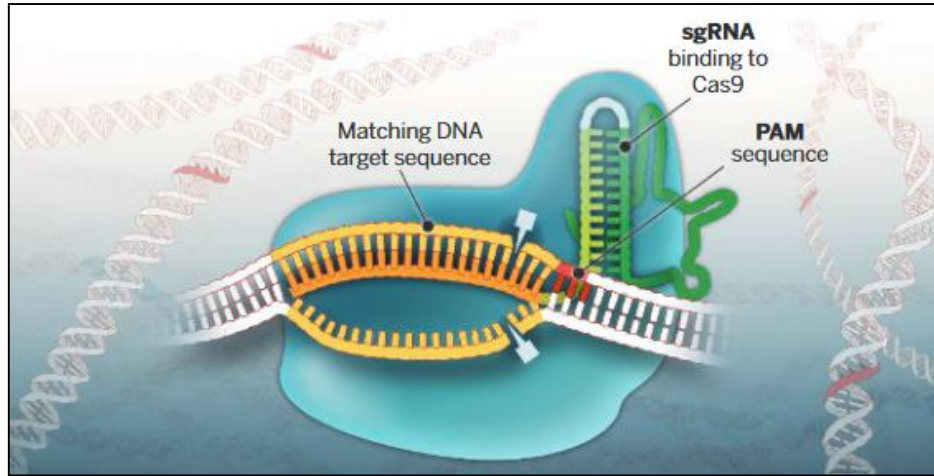


Cas9 CAN FUNCTION AS AN RNA-PROGRAMMABLE ENDONUCLEASE GUIDED BY A SYNTHETIC GUIDE RNA (sgRNA)



Source: Doudna and Charpentier (2014) *Science*, 346.

CONSEQUENCES OF CRISPR-Cas9 DNA CLEAVAGE



Source: *Molecular Biology*, Craig *et al.*

Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype

Hao Yin^{1,9}, Wen Xue^{1,9}, Sidi Chen¹, Roman L Bogorad¹, Eric Benedetti², Markus Grompe², Victor Koteliensky³, Phillip A Sharp^{1,4}, Tyler Jacks^{1,4,5} & Daniel G Anderson^{1,6-8}

Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA

Chengzu Long,^{1*} John R. McAnally,^{1*} John M. Shelton,² Alex A. Mireault,¹ Rhonda Bassel-Duby,¹ Eric N. Olson^{1†}

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,^{1,5,7} Bin Shen,^{2,7} Yiqiang Cui,^{3,7} Yongchang Chen,^{1,5,7} Jianying Wang,² Lei Wang,³ Yu Kang,^{1,5} Xiaoyang Zhao,⁴ Wei Si,^{1,5} Wei Li,⁴ Andy Peng Xiang,⁹ Jiankui Zhou,² Xuejiang Guo,³ Ye Bi,³ Chenyang Si,^{1,5} Bian Hu,² Guoying Dong,³ Hong Wang,^{1,5} Zuomin Zhou,³ Tianqing Li,^{1,5} Tao Tan,^{1,5} Xiuqiong Pu,^{1,5} Fang Wang,^{1,5} Shaohui Ji,^{1,5} Qi Zhou,⁴ Xingxu Huang,^{2,*} Weizhi Ji,^{1,5,*} and Jiahao Sha^{3,*}

The CRISPR/Cas9 system inactivates latent HIV-1 proviral DNA

Weijun Zhu^{1*}, Rongyue Lei¹, Yann Le Duff^{2,3}, Jian Li¹, Fei Guo¹, Mark A Wainberg^{2,3} and Chen Liang^{2,3*}

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

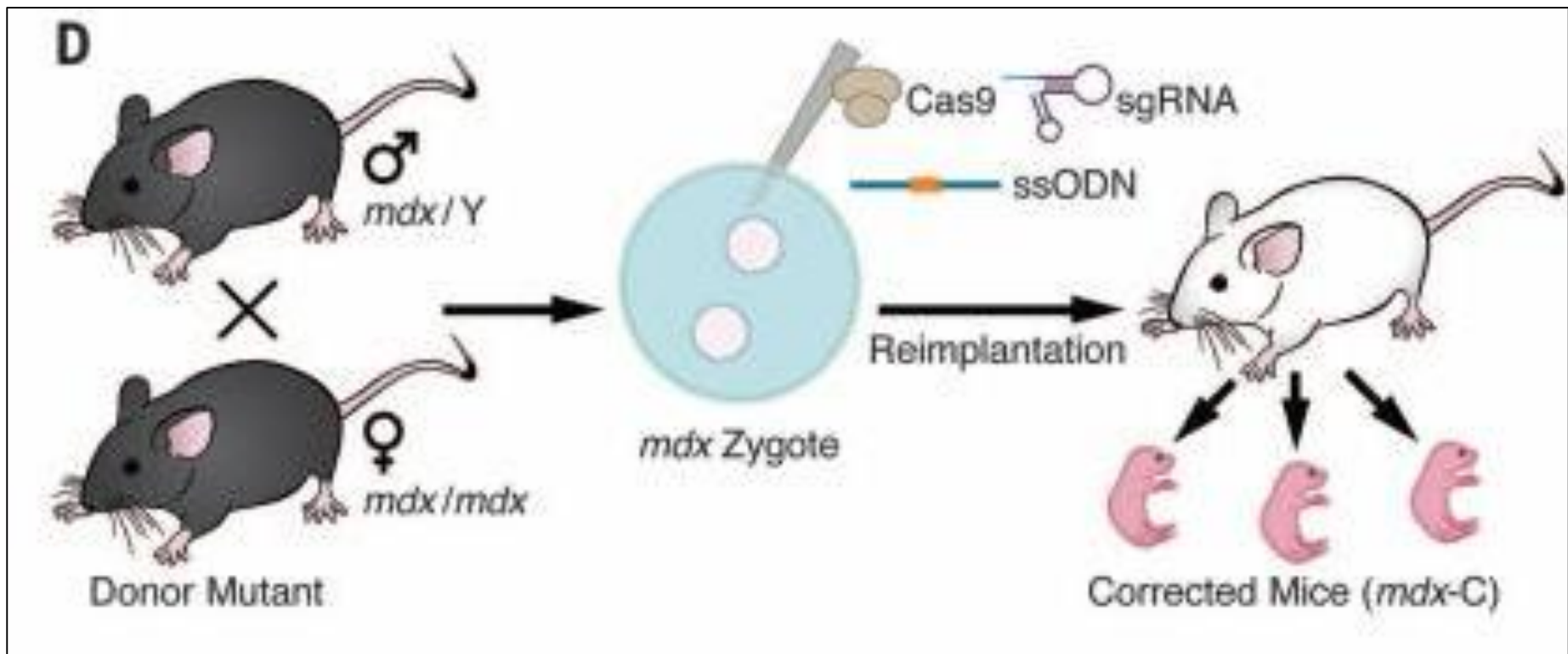
Lichun Tang^{1,2} · Yanting Zeng³ · Hongzi Du³ · Mengmeng Gong¹ · Jin Peng¹ · Buxi Zhang¹ · Ming Lei³ · Fang Zhao⁴ · Weihua Wang⁵ · Xiaowei Li⁶ · Jianqiao Liu³

Correction of a pathogenic gene mutation in human embryos

Hong Ma^{1*}, Nuria Marti-Gutierrez^{1*}, Sang-Wook Park^{2*}, Jun Wu^{3*}, Yeonmi Lee¹, Keiichiro Suzuki³, Amy Koski¹, Dongmei Ji¹, Tomonari Hayama¹, Riffat Ahmed¹, Hayley Darby¹, Crystal Van Dyken¹, Ying Li¹, Eunju Kang¹, A.-Reum Park², Daesik Kim⁴, Sang-Tae Kim², Jianhui Gong^{5,6,7,8}, Ying Gu^{5,6,7}, Xun Xu^{5,6,7}, David Battaglia^{1,9}, Sacha A. Krieg⁹, David M. Lee⁹, Diana H. Wu⁹, Don P. Wolf¹, Stephen B. Heitner¹⁰, Juan Carlos Izpisua Belmonte^{3§}, Paula Amato^{1,9§}, Jin-Soo Kim^{2,4§}, Sanjiv Kaul^{10§} & Shoukhrat Mitalipov^{1,10§}

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Long *et al.* (2014) *Science*, 345; 1184 – 1188.

POTENTIAL PROBLEMS WITH CRISPR/CAS9

1. Mosaicism

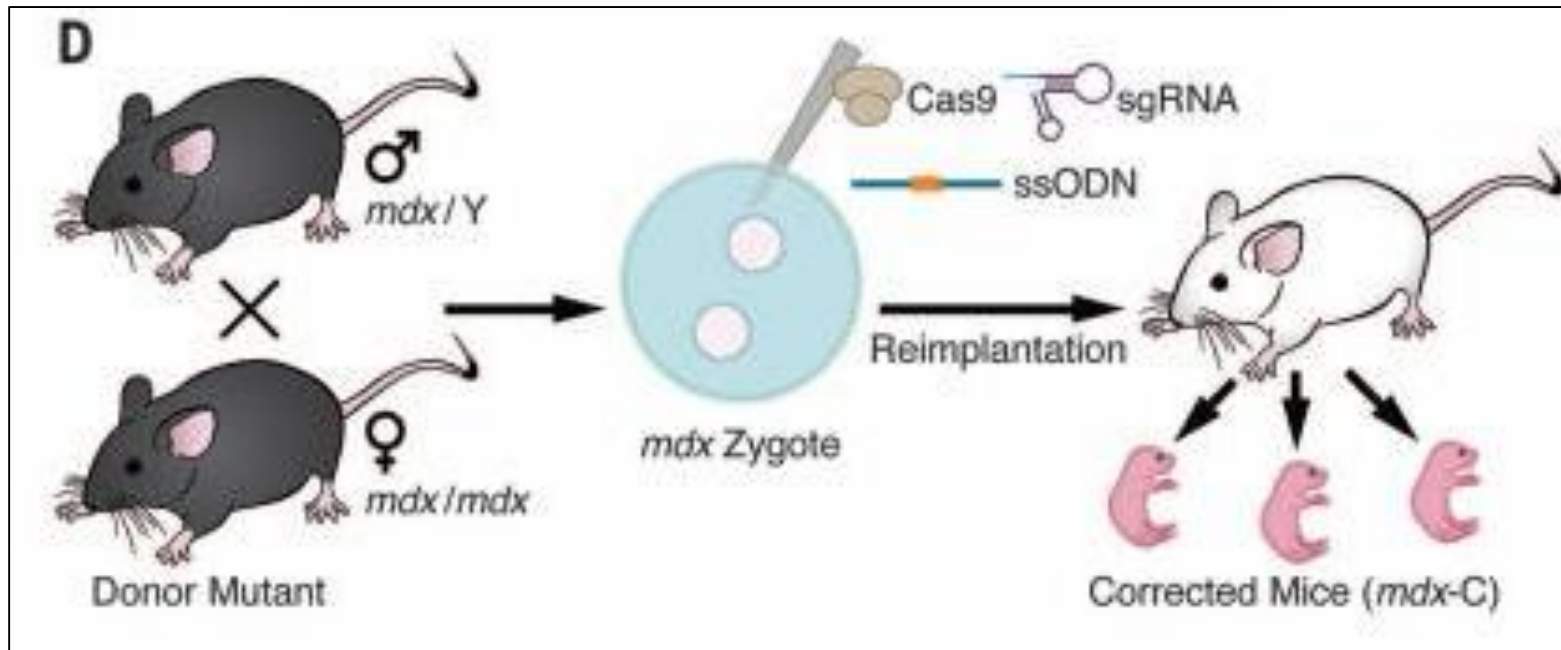


2. Off-Target Effects



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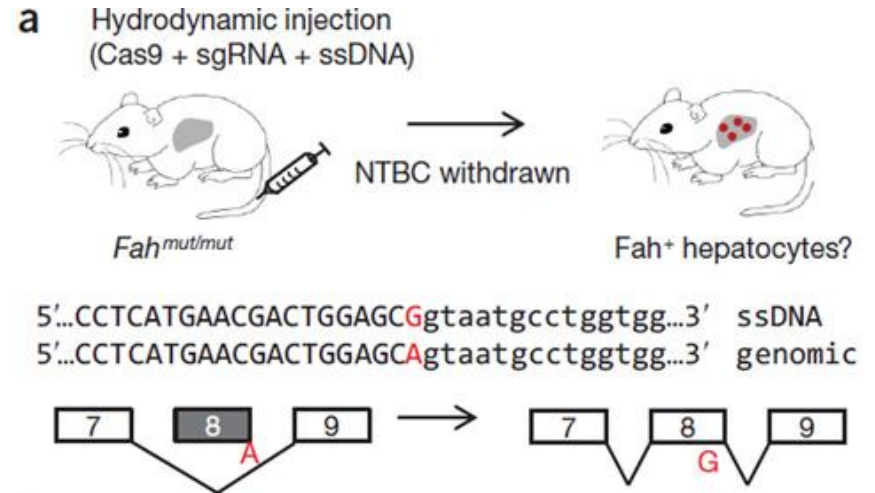
- 11 $mdx-C$ (corrected) mice analyzed
- High degree of mosaicism – 2 to 100% correction
- Muscle rescue exceeded efficiency of gene correction
- No detected off-target effects

Long *et al.* (2014) *Science*, 345; 1184 – 1188.

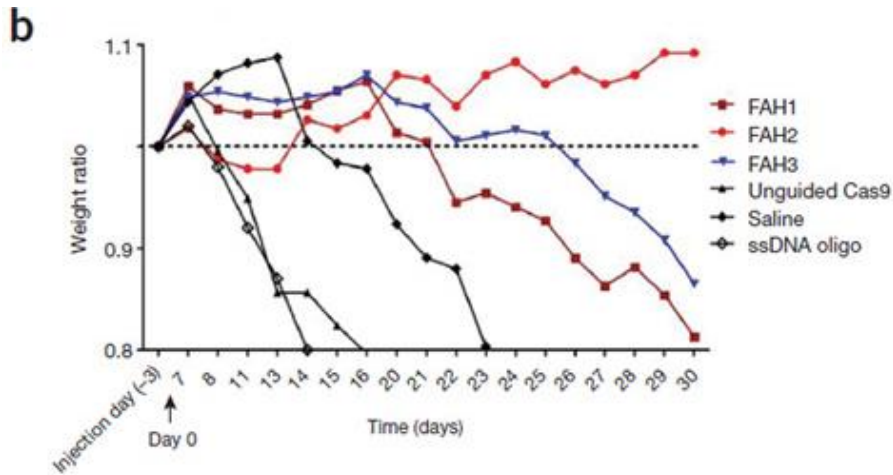
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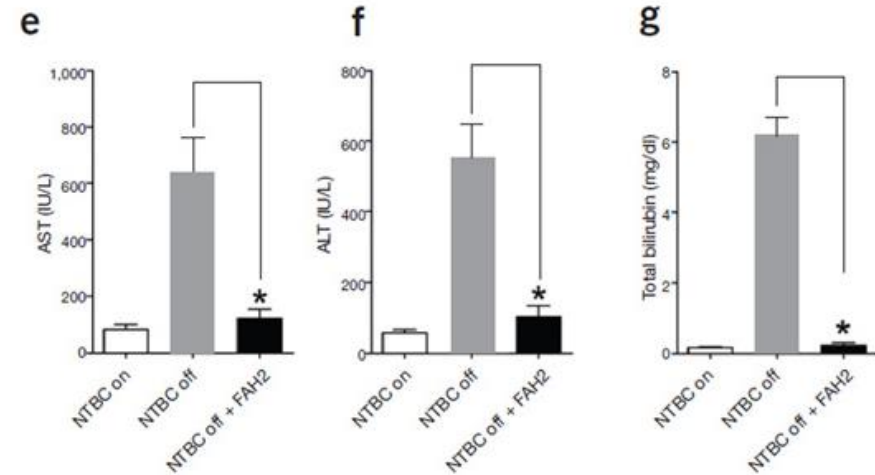
EXPERIMENTAL SET-UP



BODY WEIGHT/TIME



MARKERS OF LIVER DAMAGE



Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,^{1,5,7} Bin Shen,^{2,7} Yiqiang Cui,^{3,7} Yongchang Chen,^{1,5,7} Jianying Wang,² Lei Wang,³ Yu Kang,^{1,5} Xiaoyang Zhao,⁴ Wei Si,^{1,5} Wei Li,⁴ Andy Peng Xiang,⁶ Jiankui Zhou,² Xuejiang Guo,³ Ye Bi,³ Chenyang Si,^{1,5} Bian Hu,² Guoying Dong,³ Hong Wang,^{1,5} Zuomin Zhou,³ Tianqing Li,^{1,5} Tao Tan,^{1,5} Xiuqiong Pu,^{1,5} Fang Wang,^{1,5} Shaohui Ji,^{1,5} Qi Zhou,⁴ Xingxu Huang,^{2,*} Weizhi Ji,^{1,5,*} and Jiahao Sha^{3,*}

- Two genes targeted at once
- Genes – Ppar- γ ; Rag-1
- Experimental Set-up – Inject Cas9 mRNA and sgRNAs into one-cell embryos to mutate genes
- Transfer embryos to surrogate mothers

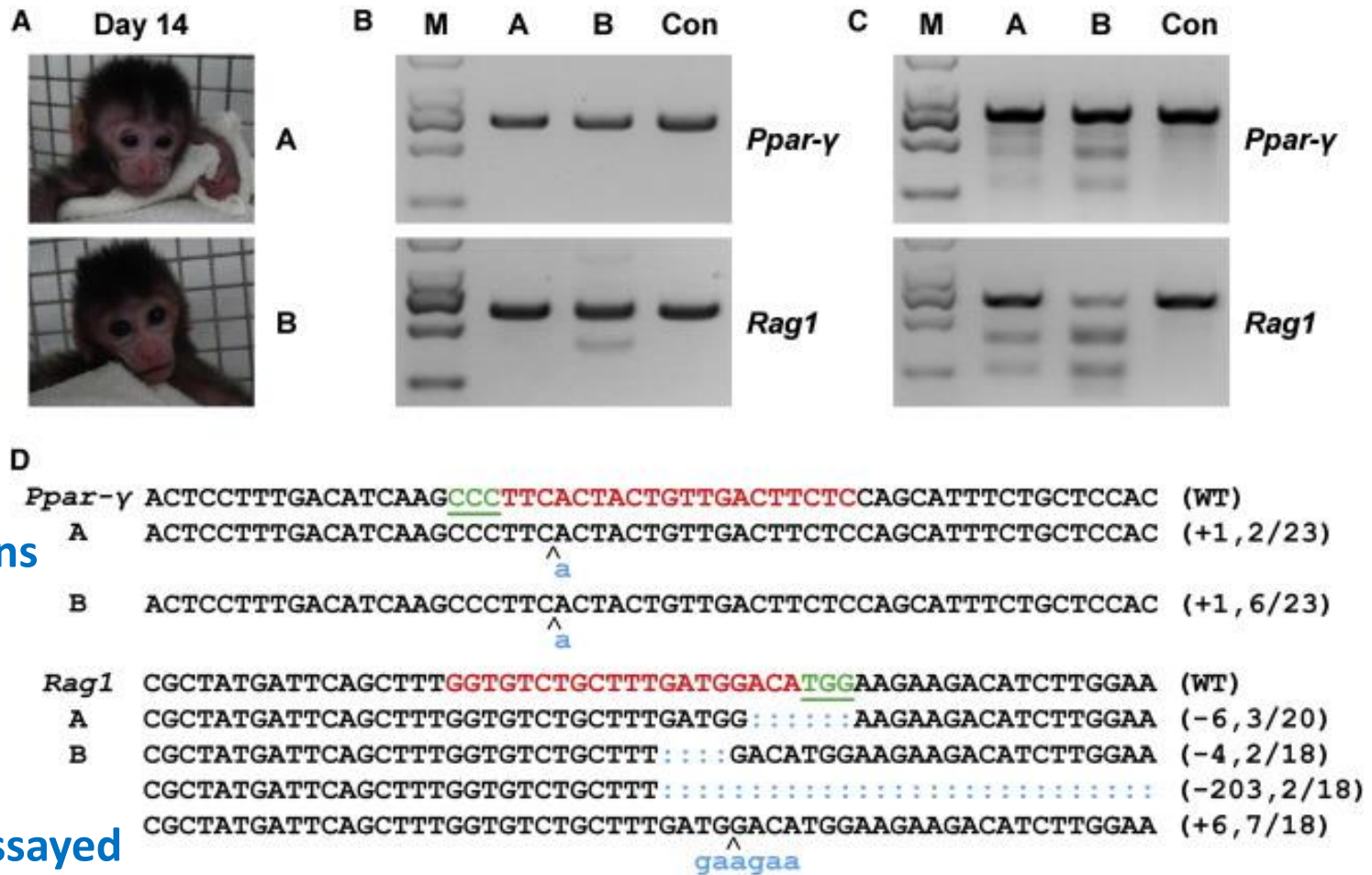


Niu et al., Cell

Twin cynomolgus monkeys born in China are the first with mutations in specific target genes.

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

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Cas9/sgRNA induced Mutations

No detected off-target effects


Mosaicism not assayed at time of publication

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

Lichun Tang^{1,2}  · Yanting Zeng³ · Hongzi Du³ · Mengmeng Gong¹ · Jin Peng¹ · Buxi Zhang¹ · Ming Lei³ · Fang Zhao⁴ · Weihua Wang⁵ · Xiaowei Li⁶ · Jianqiao Liu³

- Patients undergoing IVF treatment at Center for Reproductive Medicine (Guangzhou, China)
- Normal diploid zygotes used for study (informed consent of patients)
- Attempts were made to correct two mutations using CRISPR/Cas9
 - Mutation in β -globin gene that causes thalassemia
 - Mutation in G6PD gene that causes anemia
- Sperm carrying mutations injected into oocytes to generate zygotes
- Cas9 protein, sgRNAs, and ssDNA were injected into zygotes
- Embryos were cultured for two days and then “harvested” for analyses

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

Lichun Tang^{1,2}  · Yanting Zeng³ · Hongzi Du³ · Mengmeng Gong¹ · Jin Peng¹ · Buxi Zhang¹ · Ming Lei³ · Fang Zhao⁴ · Weihua Wang⁵ · Xiaowei Li⁶ · Jianqiao Liu³

RESULTS

- Sample size very low (10 embryos for β -globin study, only 2 for G6PD)
- For β -globin study, editing efficiency was 50%, gene correction efficiency was 50%
- Additional mutations were detected in uncorrected β -globin genes
- No mention of mosaicism or off-target effects
- For G6PD study, only 2 embryos were studied, both had corrected genes
- One of two embryos was mosaic (50% cells corrected, 50% cells had an additional mutation)
- No off-target effects were detected

Source: Tang *et al.* (2017) *Mol. Genet. Genomics*, 292: 525-533.

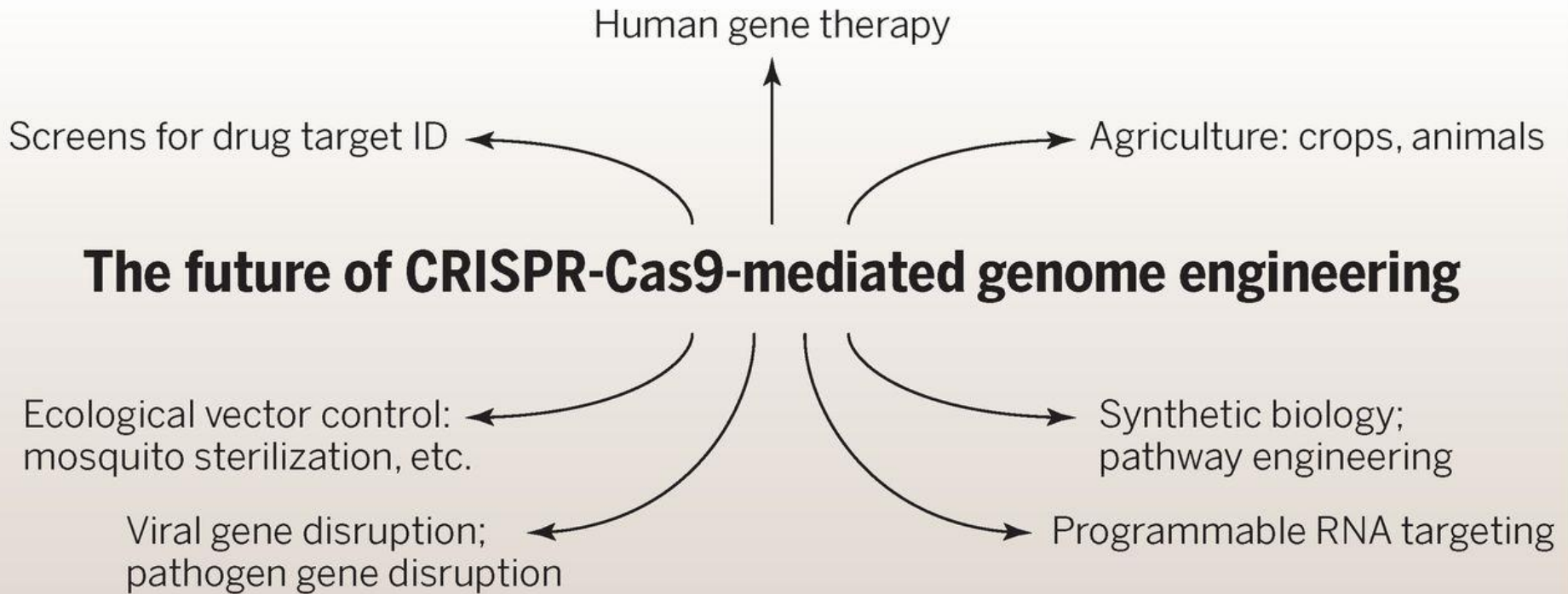


2018: First claim of CRISPR-edited babies

Sources: China News Service /VCG/Getty, npr.org




2019: 1st patient treated with CRISPR



Source: Doudna and Charpentier (2014) *Science*, 346.

SUMMARY

- 
- **CRISPR/Cas** systems function as a type of acquired immunity in bacteria
 - **Cas9** is an RNA-programmable double-stranded endonuclease
 - Double-strand breaks created by **Cas9/sgRNAs** can be repaired, changed, corrected by homologous recombination
 - Gene editing by **Cas9/sgRNAs** has been accomplished in many animals including humans
- 