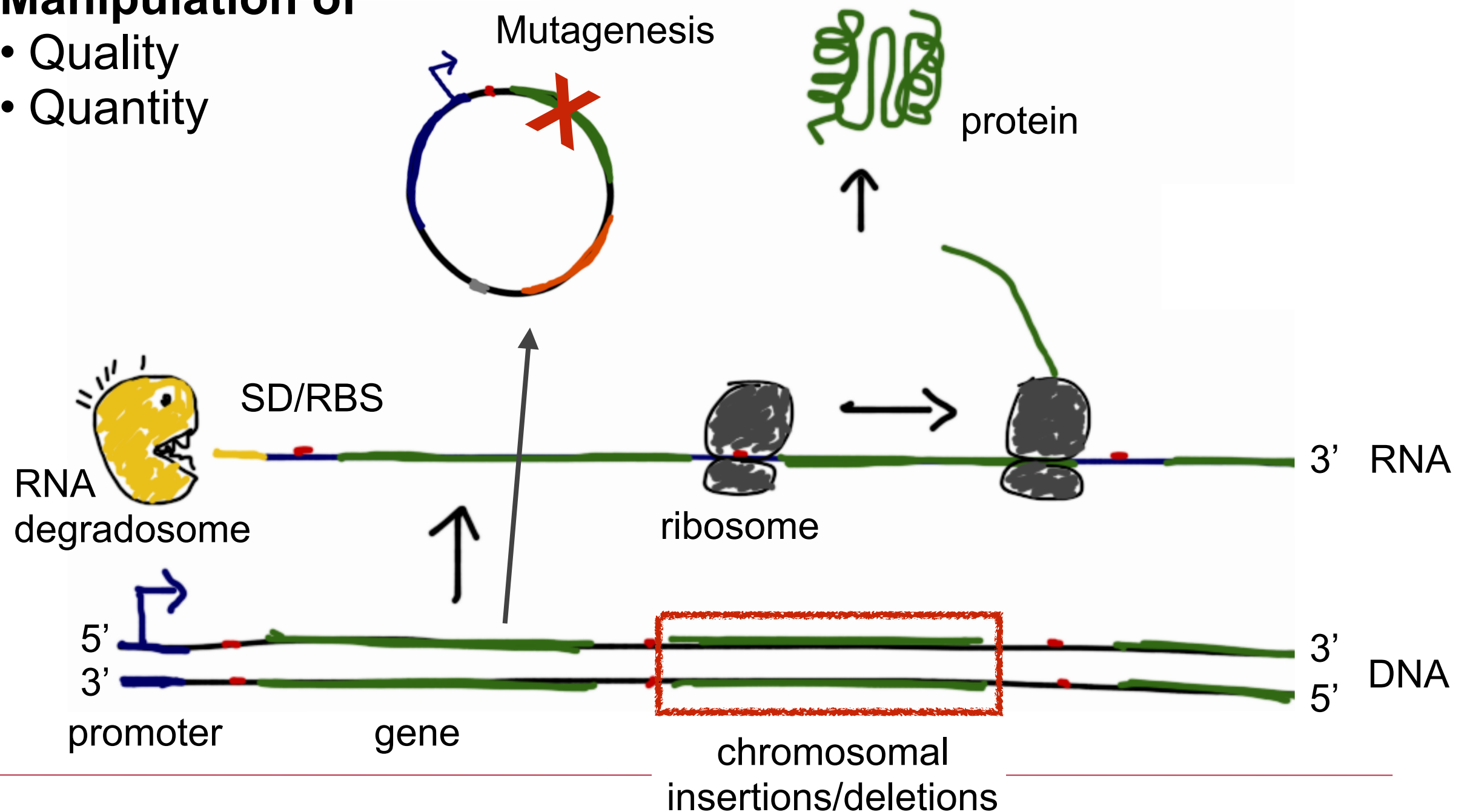


In the end it is all about making proteins... or not making

Manipulation of

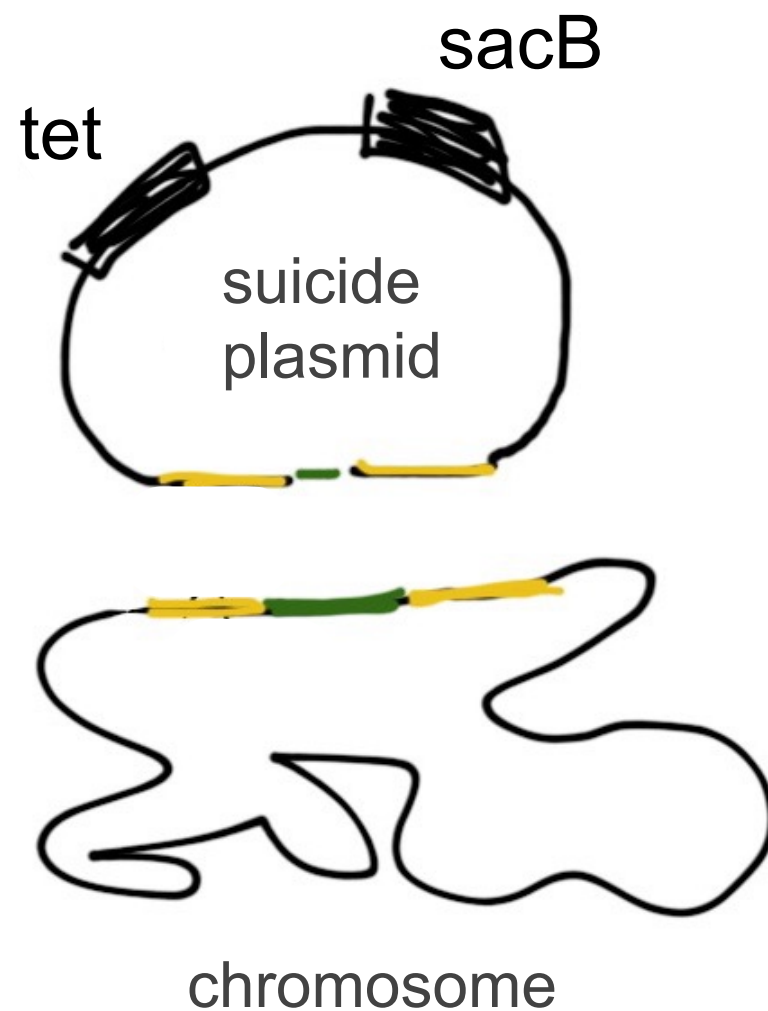
- Quality
- Quantity



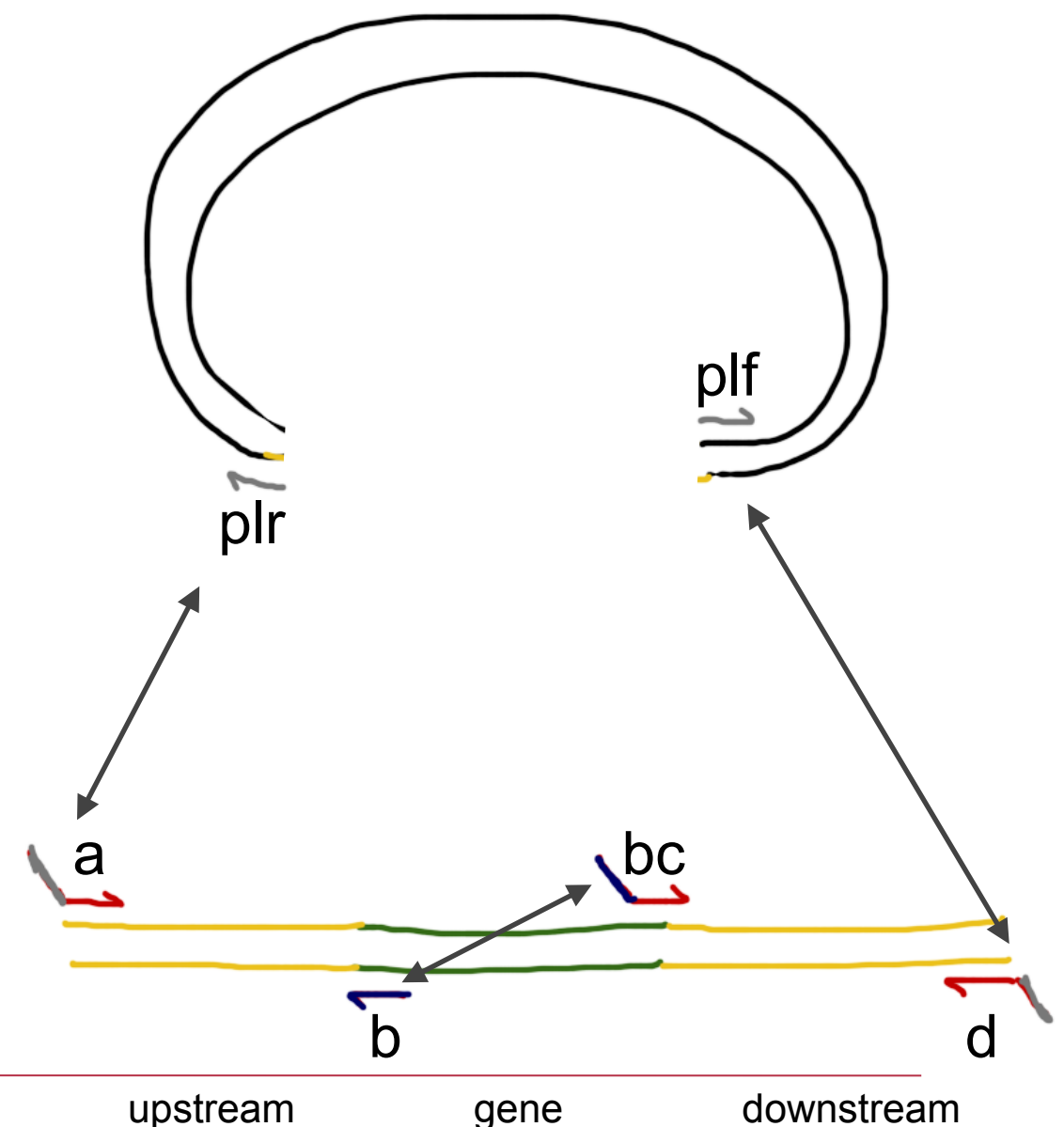
Modifying bacterial chromosomes – Allelic exchange

Using inherent homologues recombination to seamlessly exchange alleles

Making the suicide plasmid

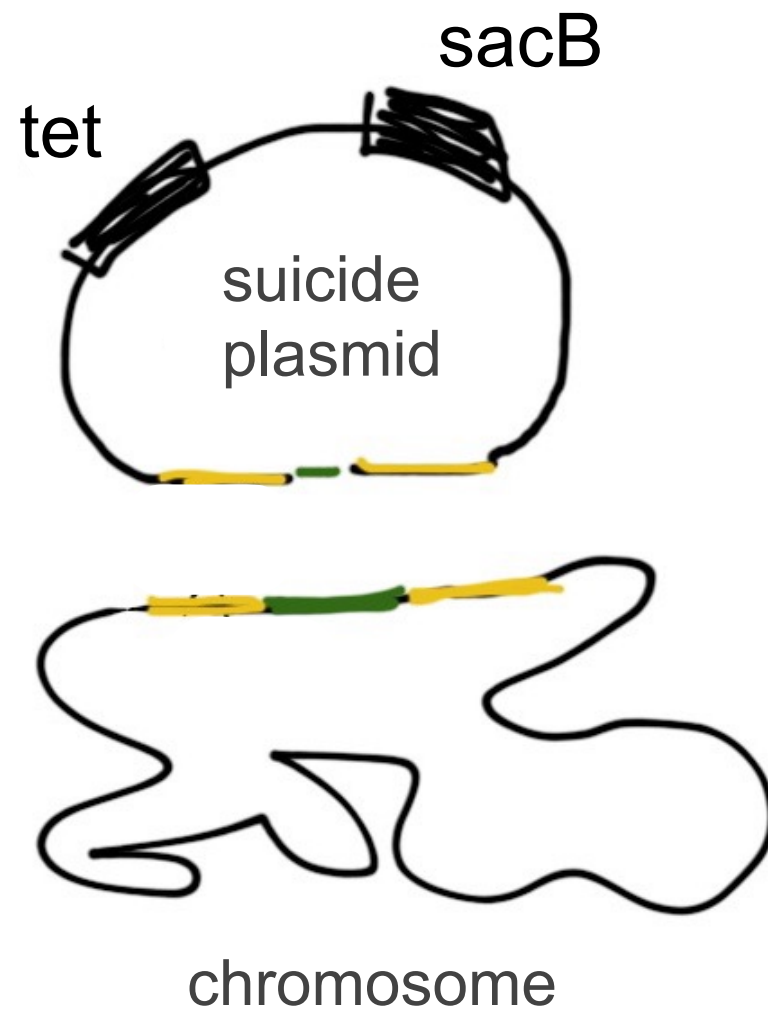


1. PCR



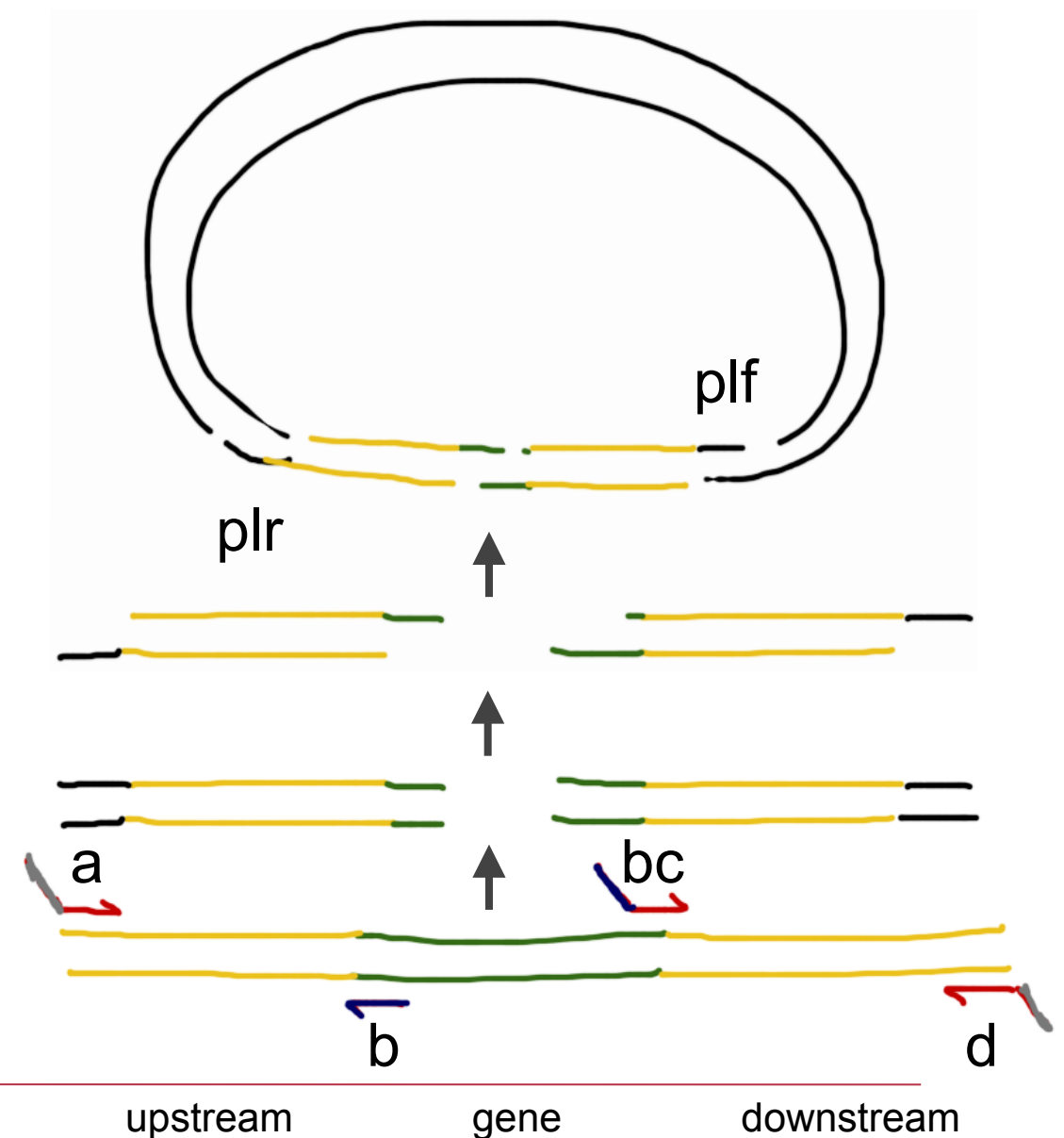
Modifying bacterial chromosomes – Allelic exchange

Making the suicide plasmid



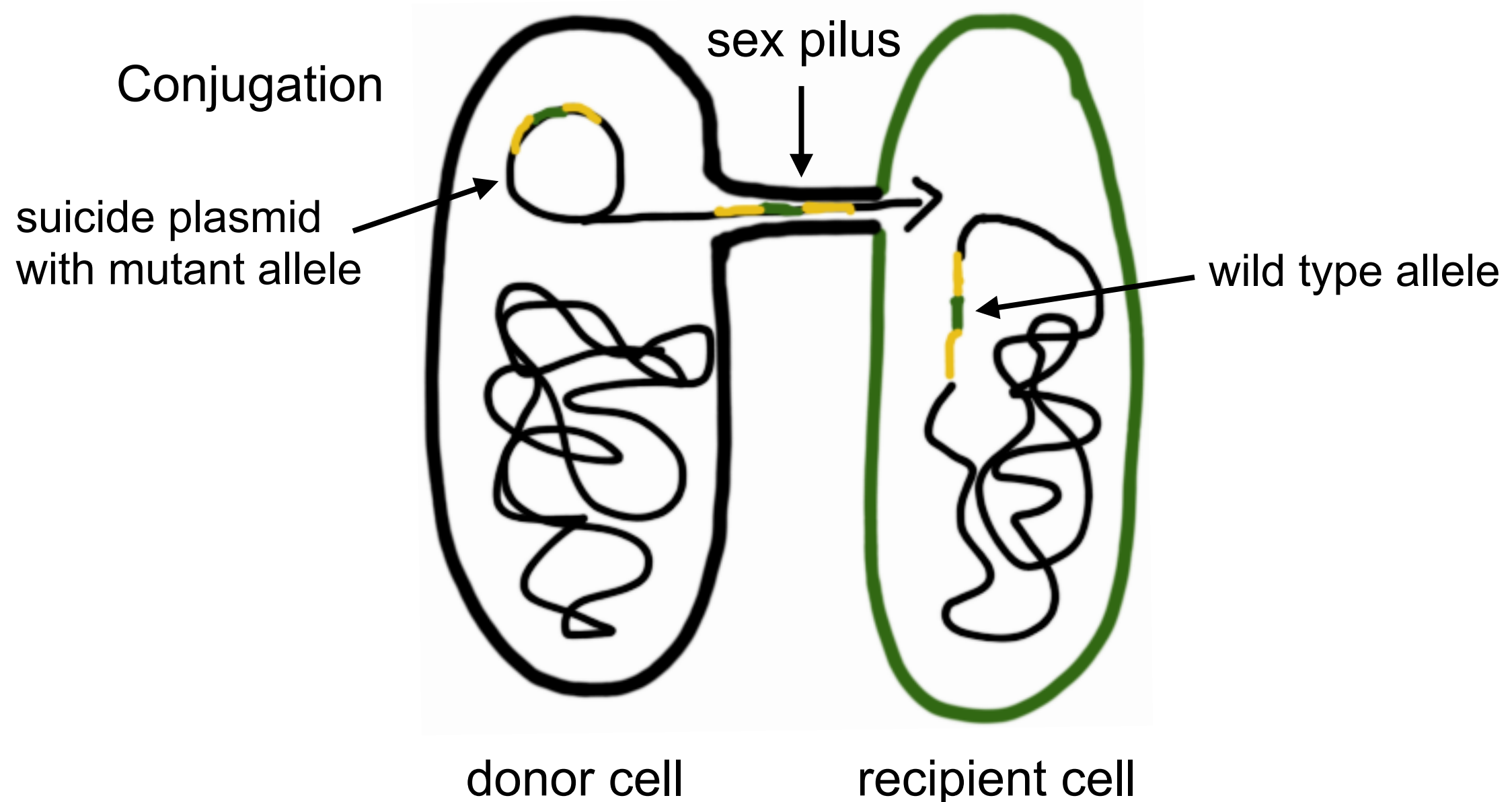
2. Gibson assembly

1. PCR

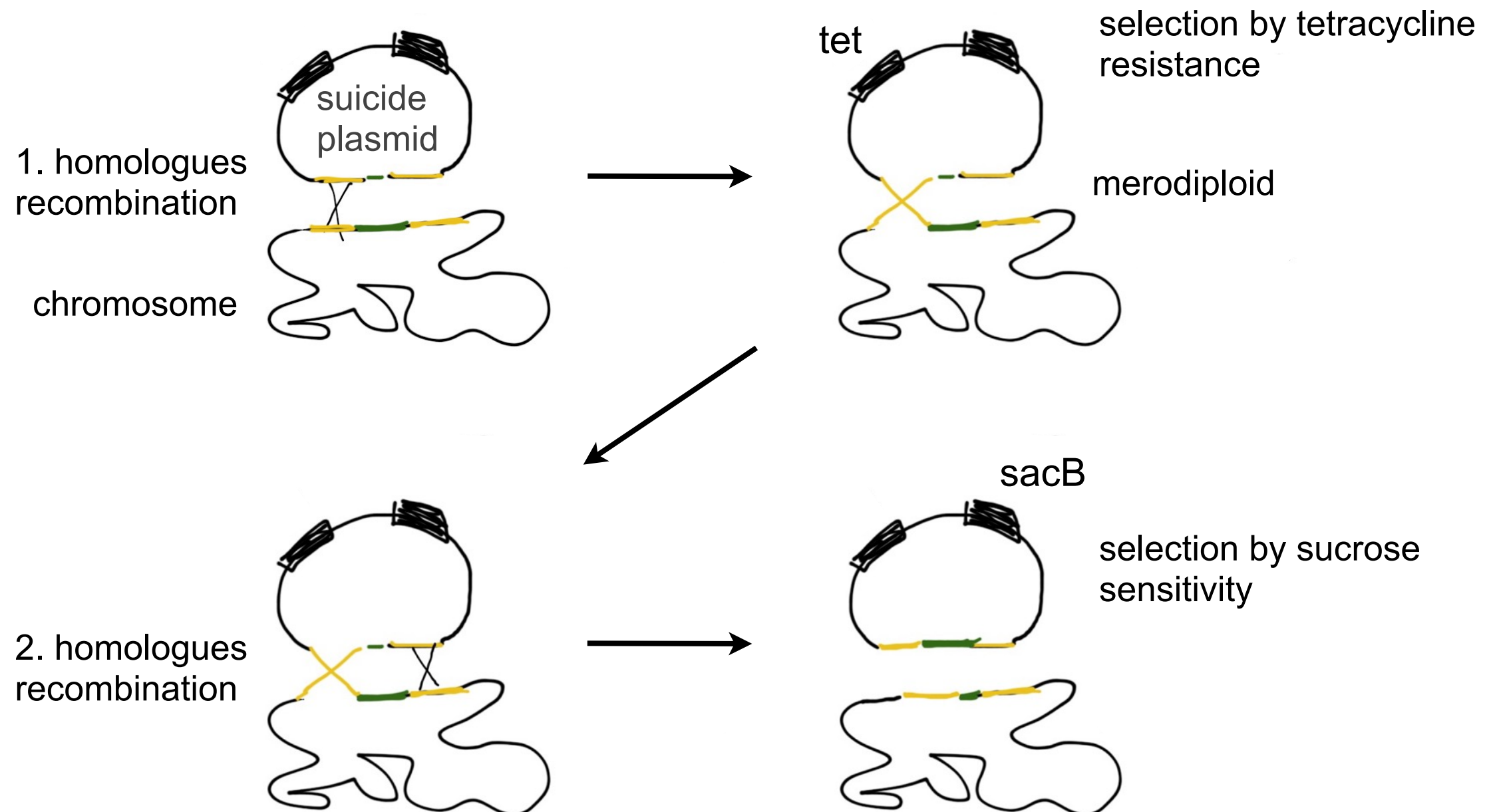


Modifying bacterial chromosomes – Allelic exchange

Mating bacteria



Modifying bacterial chromosomes – Allelic exchange



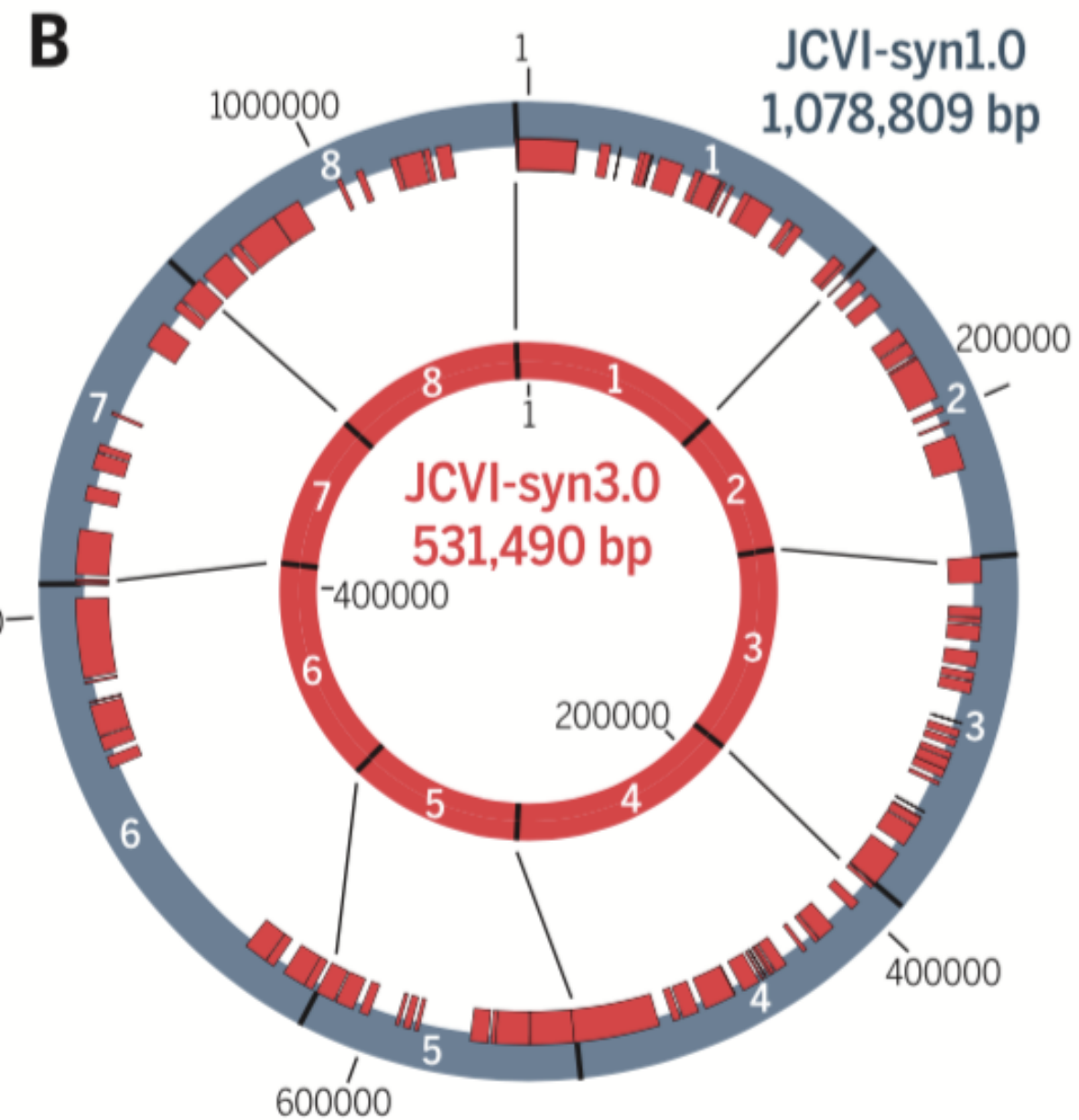
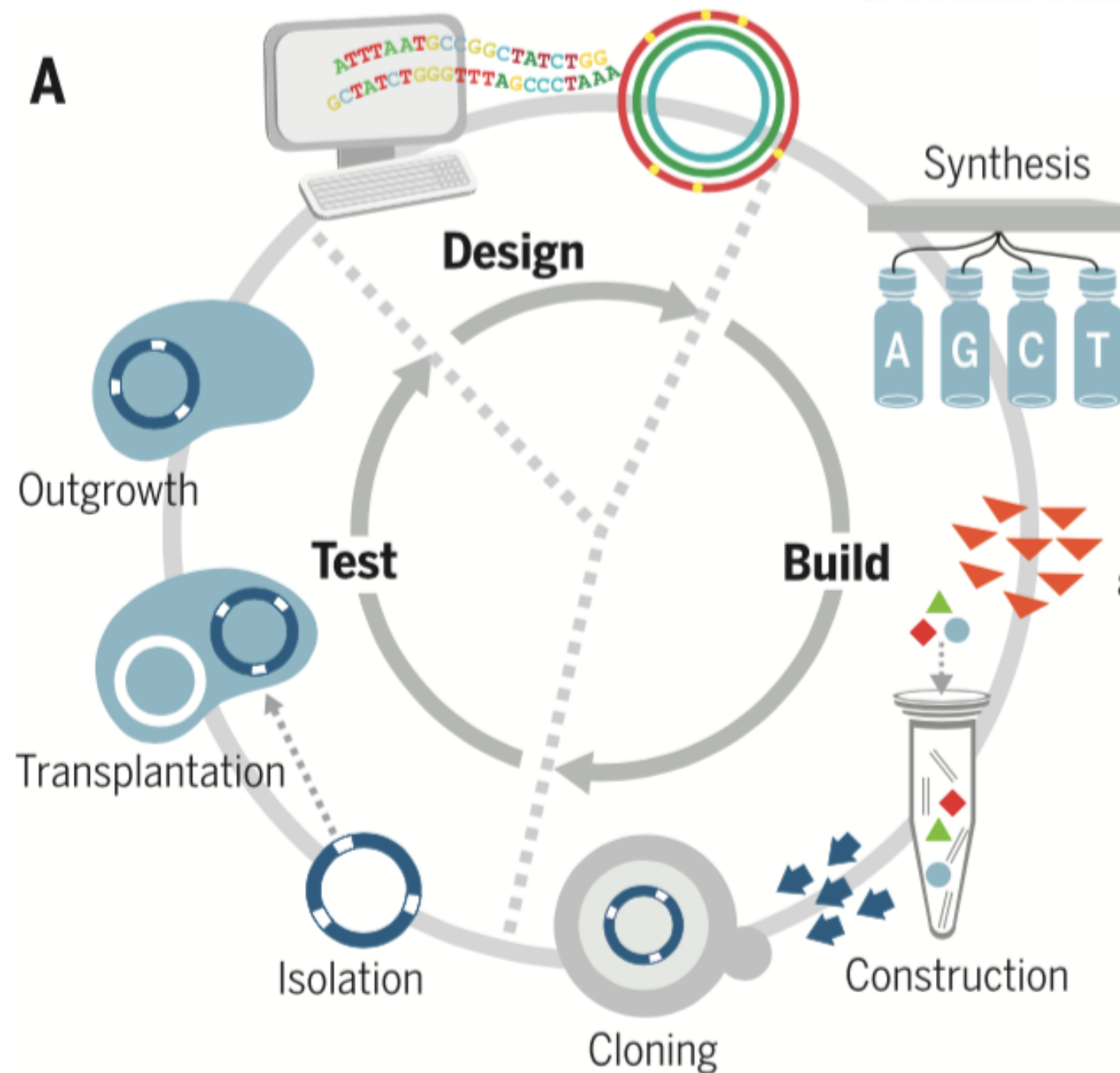
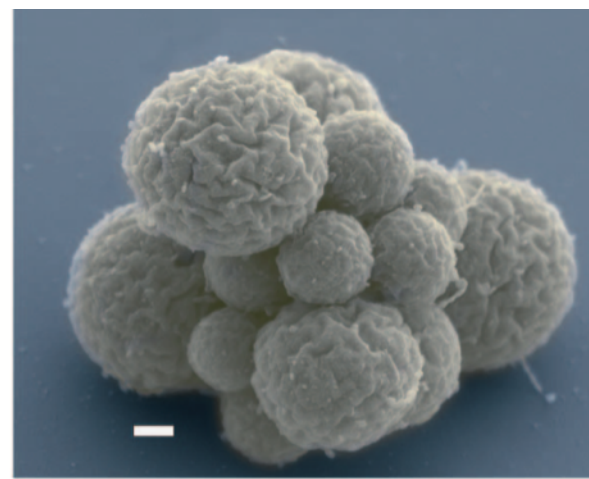


RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

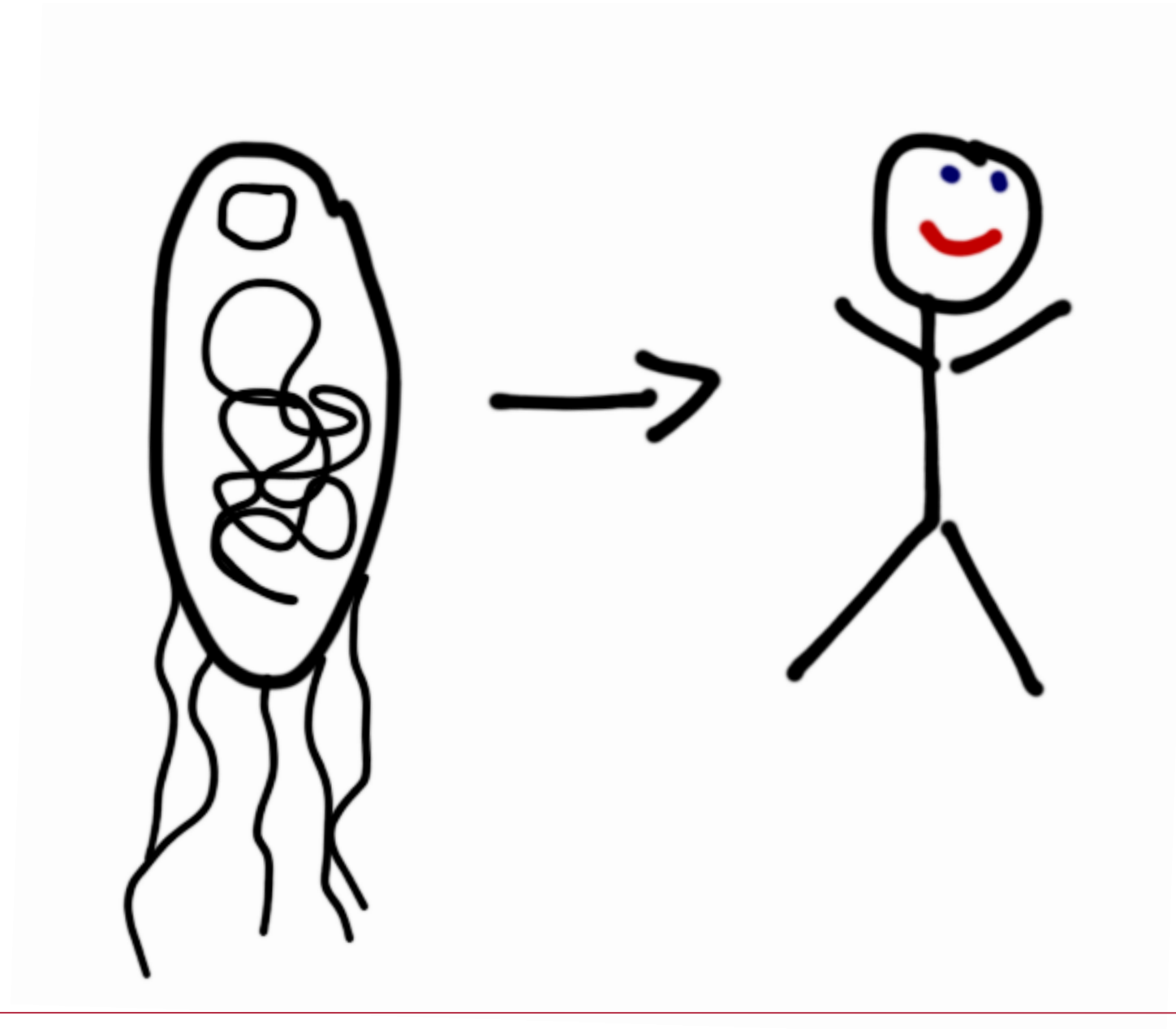
Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,^{*†} Ray-Yuan Chuang,[†] Vladimir N. Noskov, Nacyra Assad-Garcia, Thomas J. Deerinck, Mark H. Ellisman, John Gill, Krishna Kannan, Bogumil J. Karas, Li Ma, James F. Pelletier, Zhi-Qing Qi, R. Alexander Richter, Elizabeth A. Strychalski, Lijie Sun, Yo Suzuki, Billyana Tsvetanova, Kim S. Wise, Hamilton O. Smith, John I. Glass, Chuck Merryman, Daniel G. Gibson, J. Craig Venter^{*}



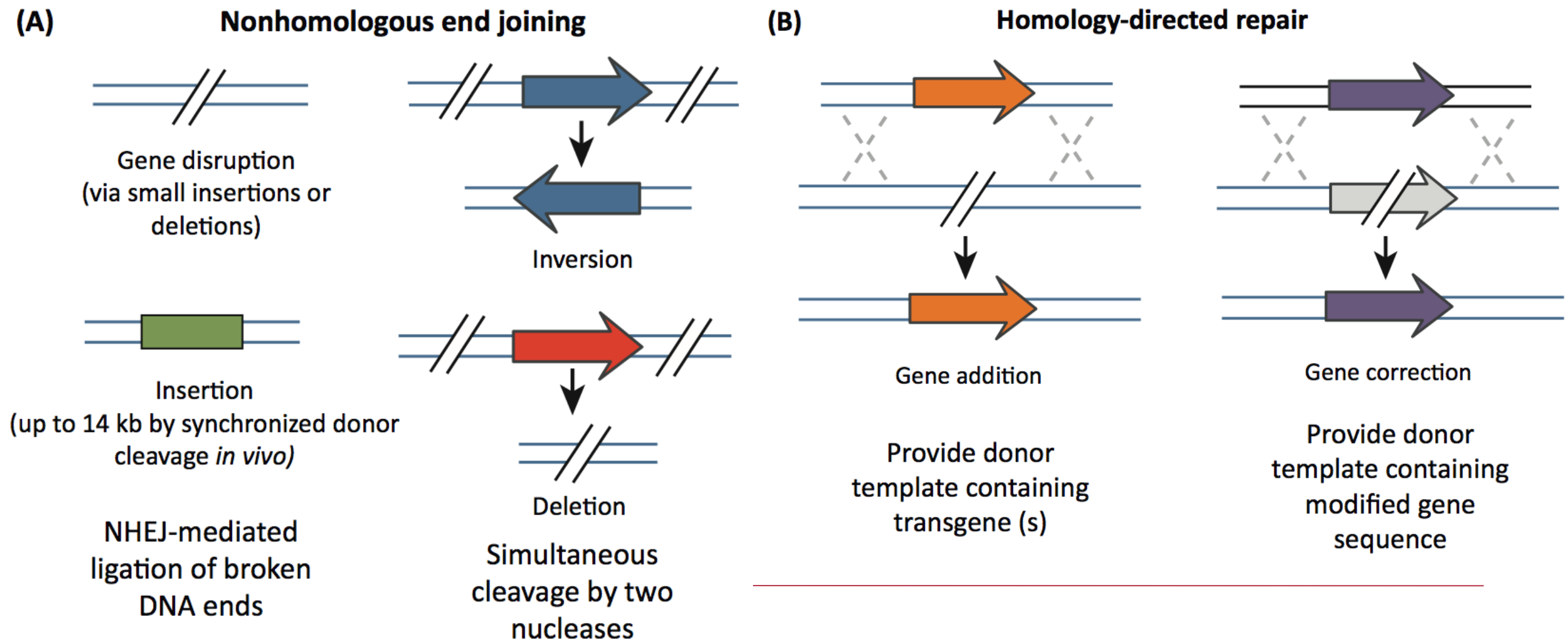


‘almost’ leaving bacteria and focusing on eukaryotes



Editing eukaryotic genomes

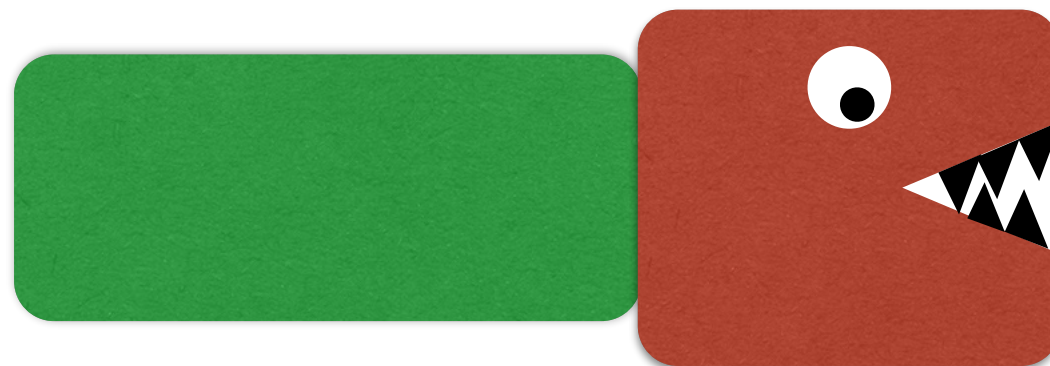
- homologues recombination is very inefficient in many eukaryotes
- introduction of **double strand breaks** activates DNA repair by either
 - **non-homologous end joining**
 - **homology-directed repair**





Engineered sequence-specific nucleases to induce targeted double-strand breaks

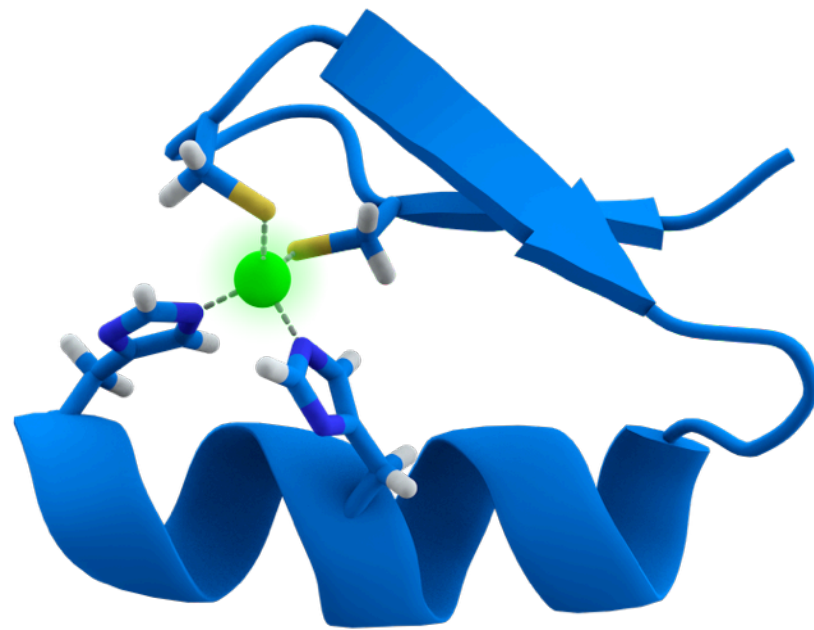
nonspecific DNA
cleavage module



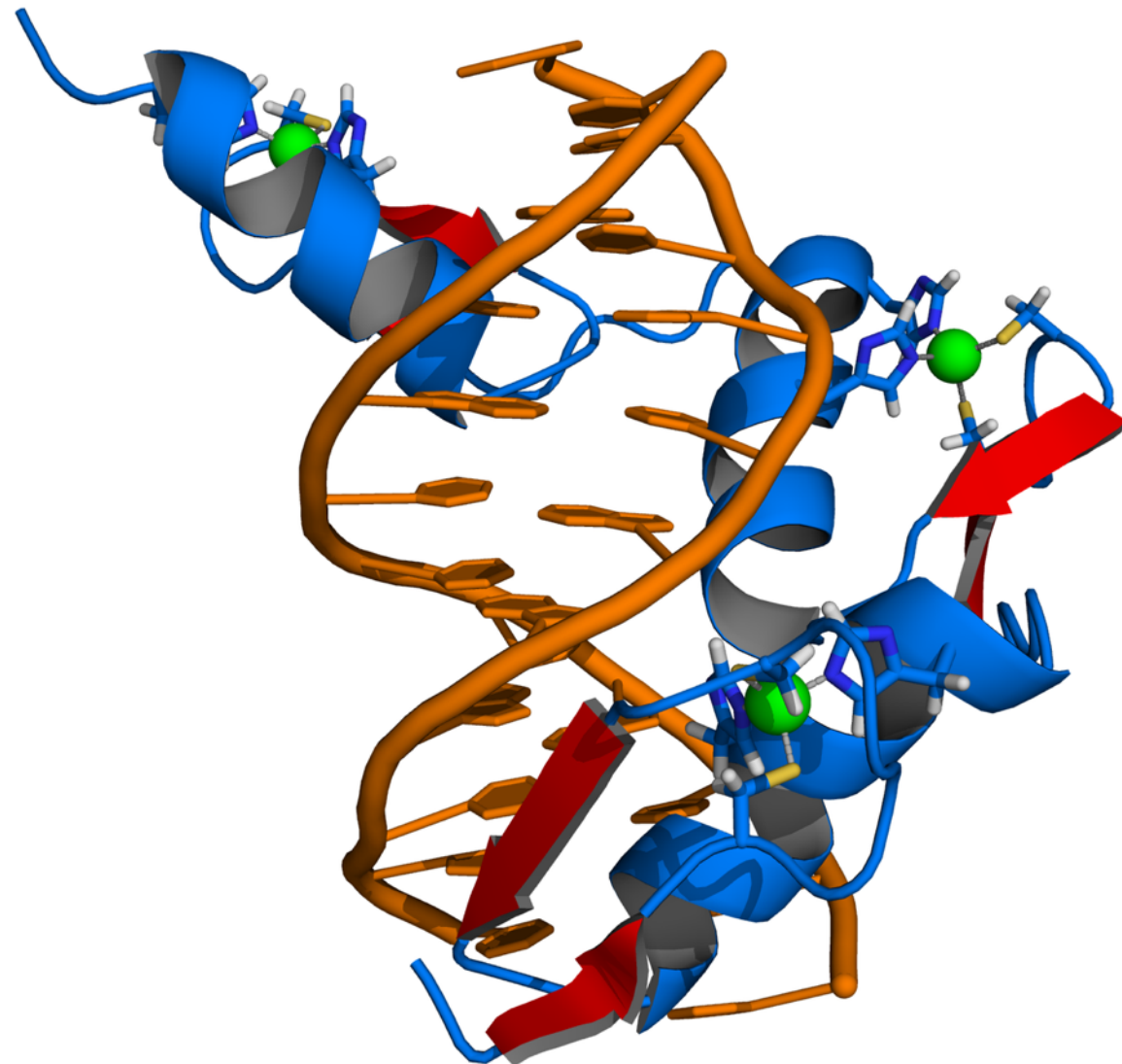
sequence-specific
DNA-binding domain

How to target a specific gene/DNA sequence?

Zinc fingers

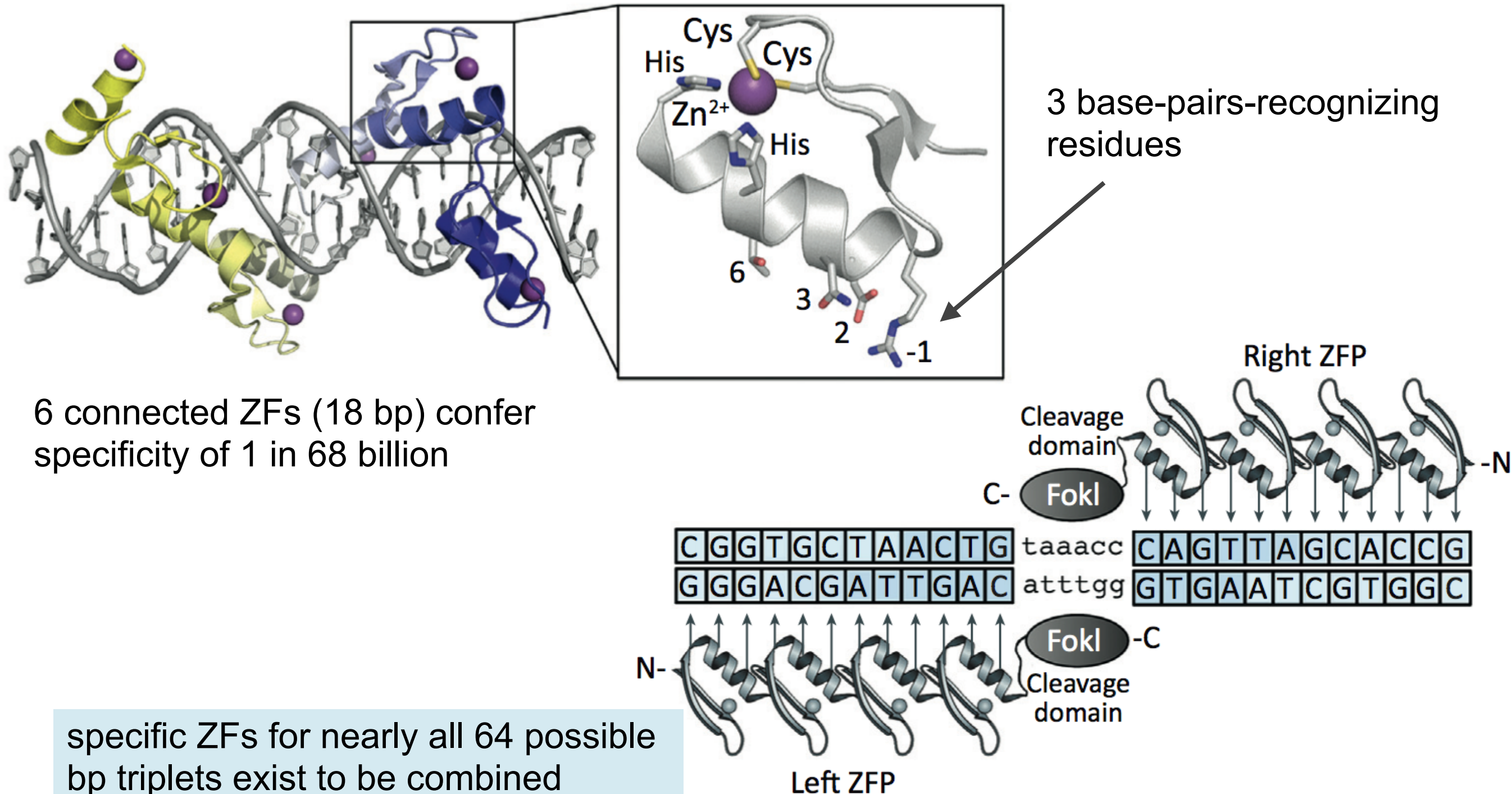


Cys₂His₂
zinc coordination



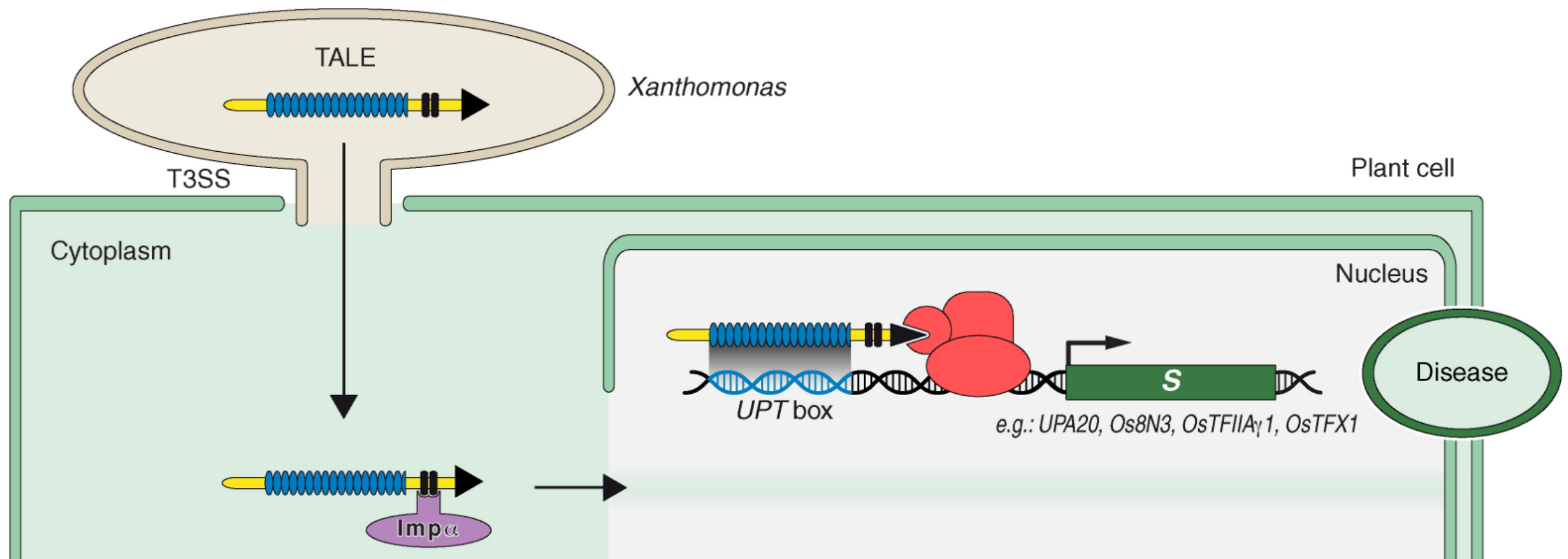
How to target a specific gene/DNA sequence?

ZFN: Zinc finger nuclea



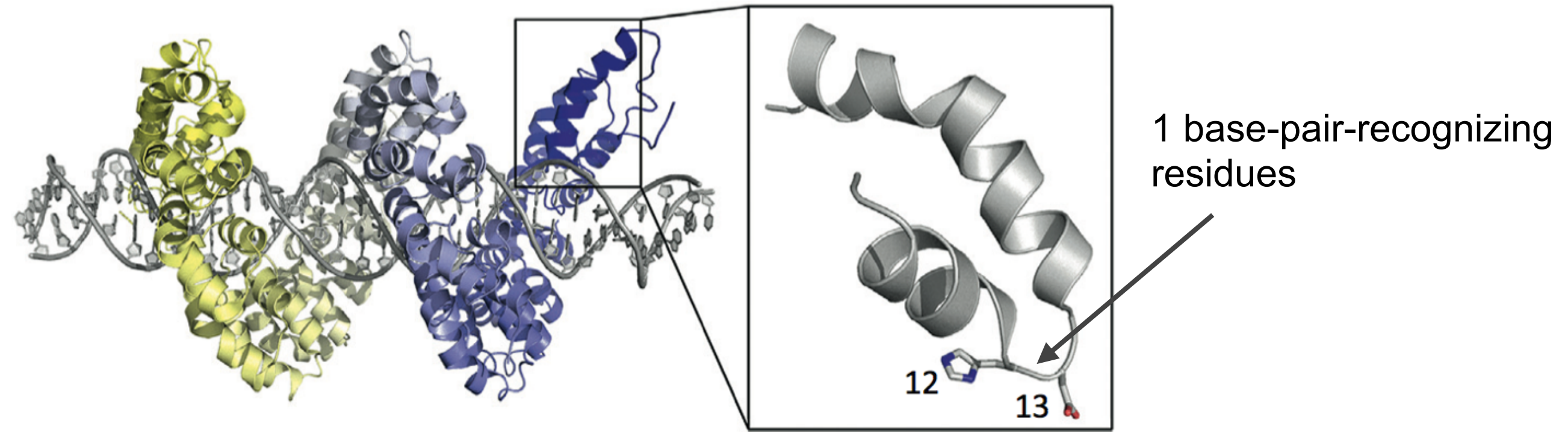
How to target a specific gene/DNA sequence?

TALE: transcription activator-like effectors

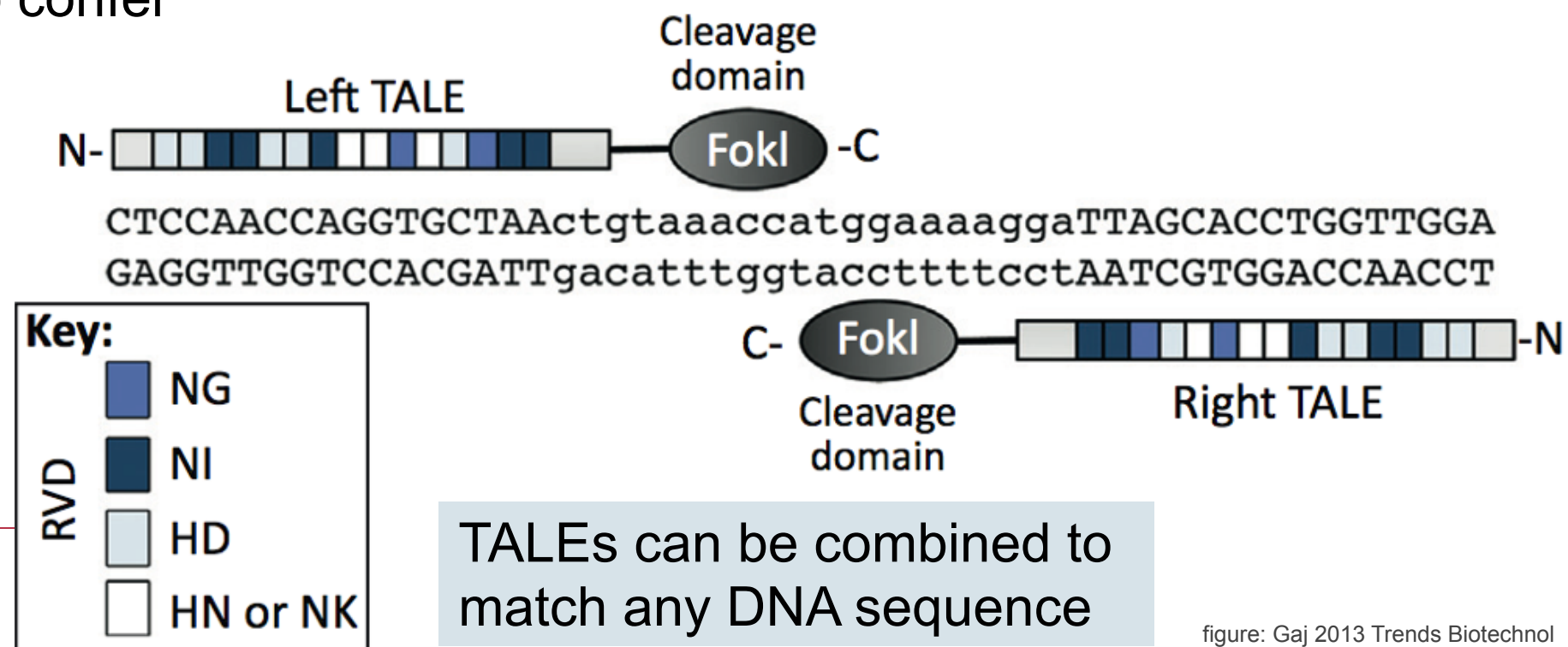


How to target a specific gene/DNA sequence?

TALEN: transcription activator-like effector nucleases



18 connected TALEs (18 bp) confer specificity of 1 in 68 billion





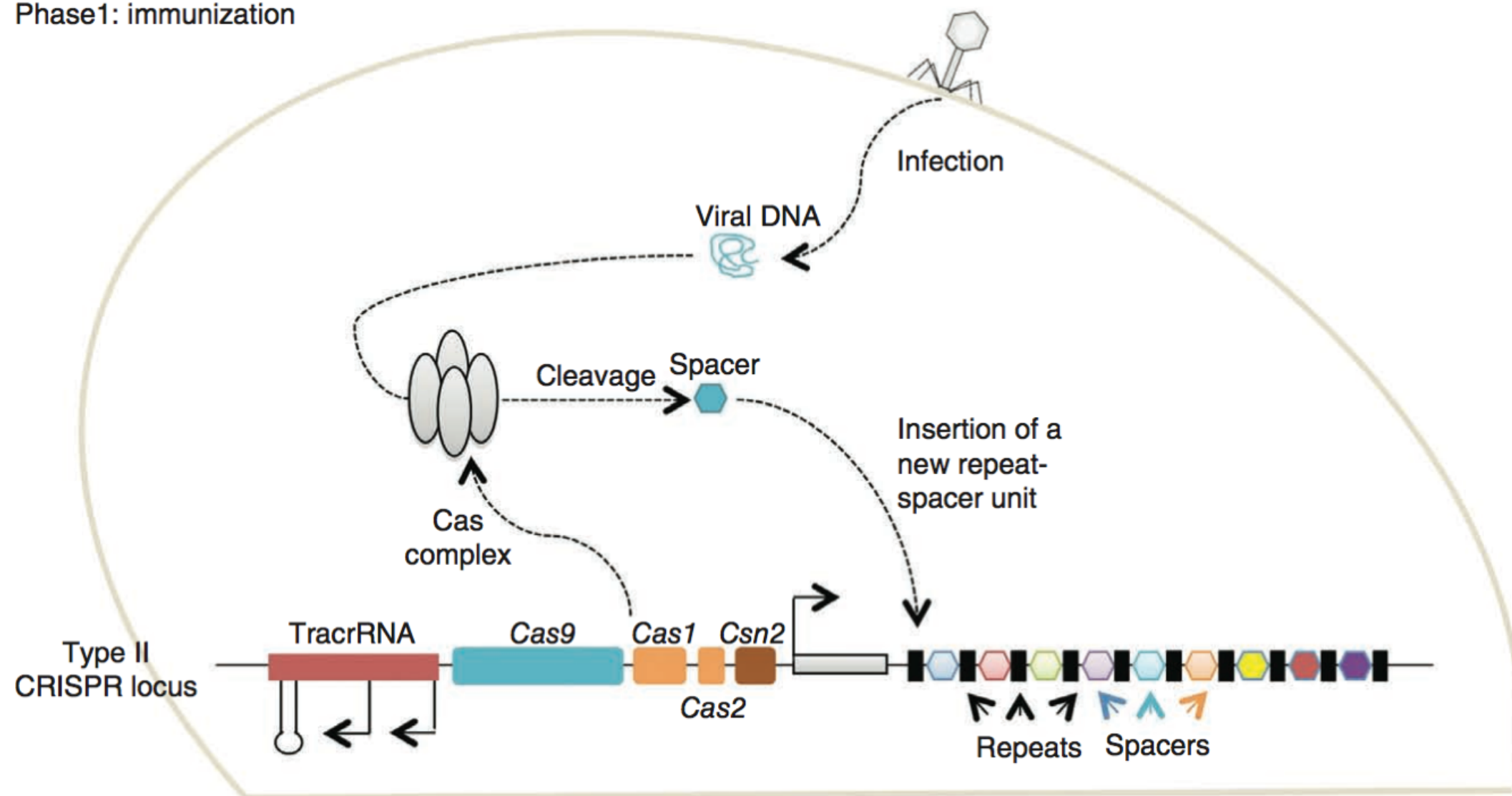
How to target a specific gene/DNA sequence?

CRISPR-Cas: clustered regularly interspaced short palindromic repeats/
CRISPR-associated

RNA-mediated DNA-targeting system that confers immunity in bacteria

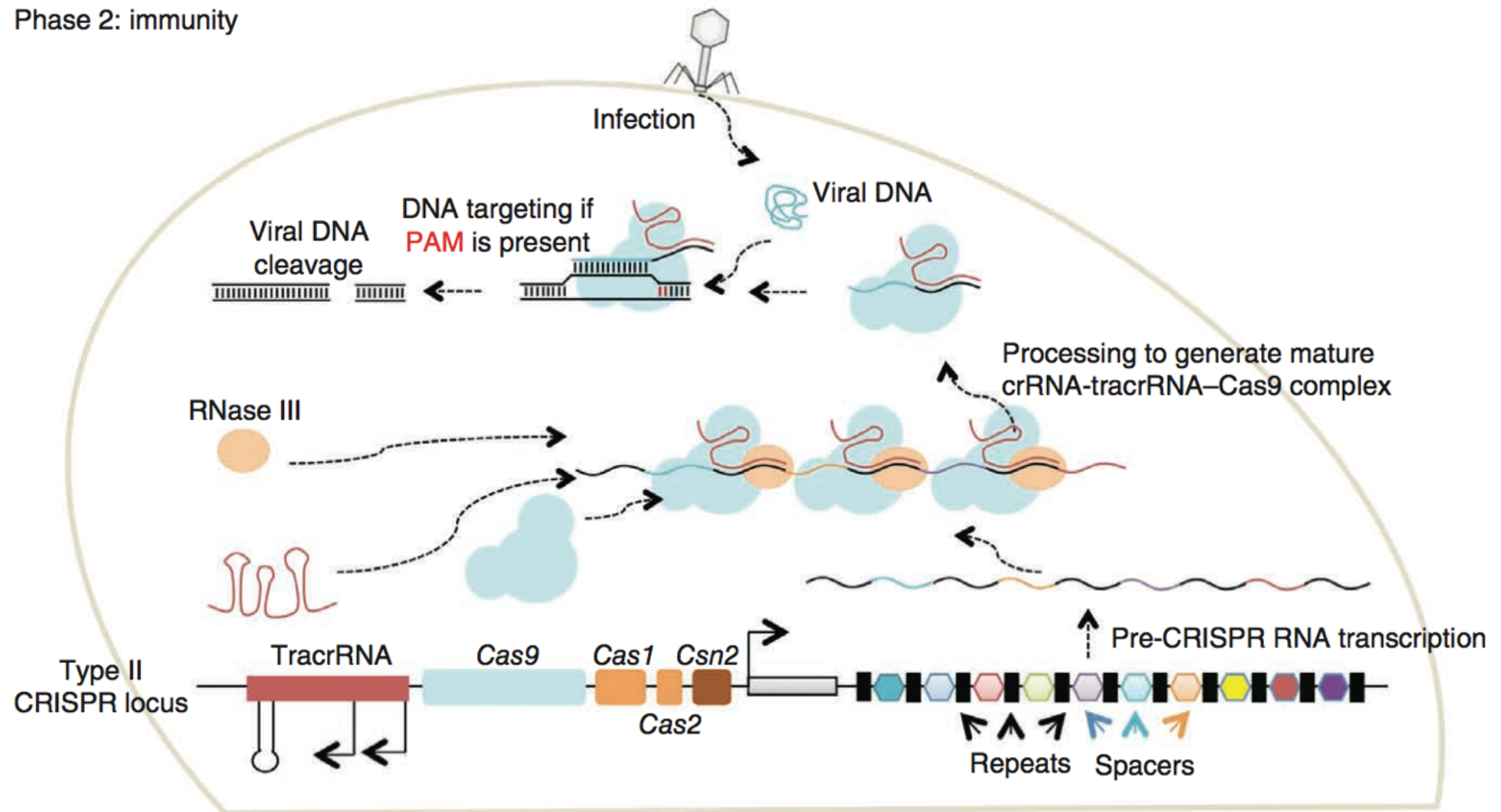
CRISPR-Cas - basics: immunization

Phase1: immunization

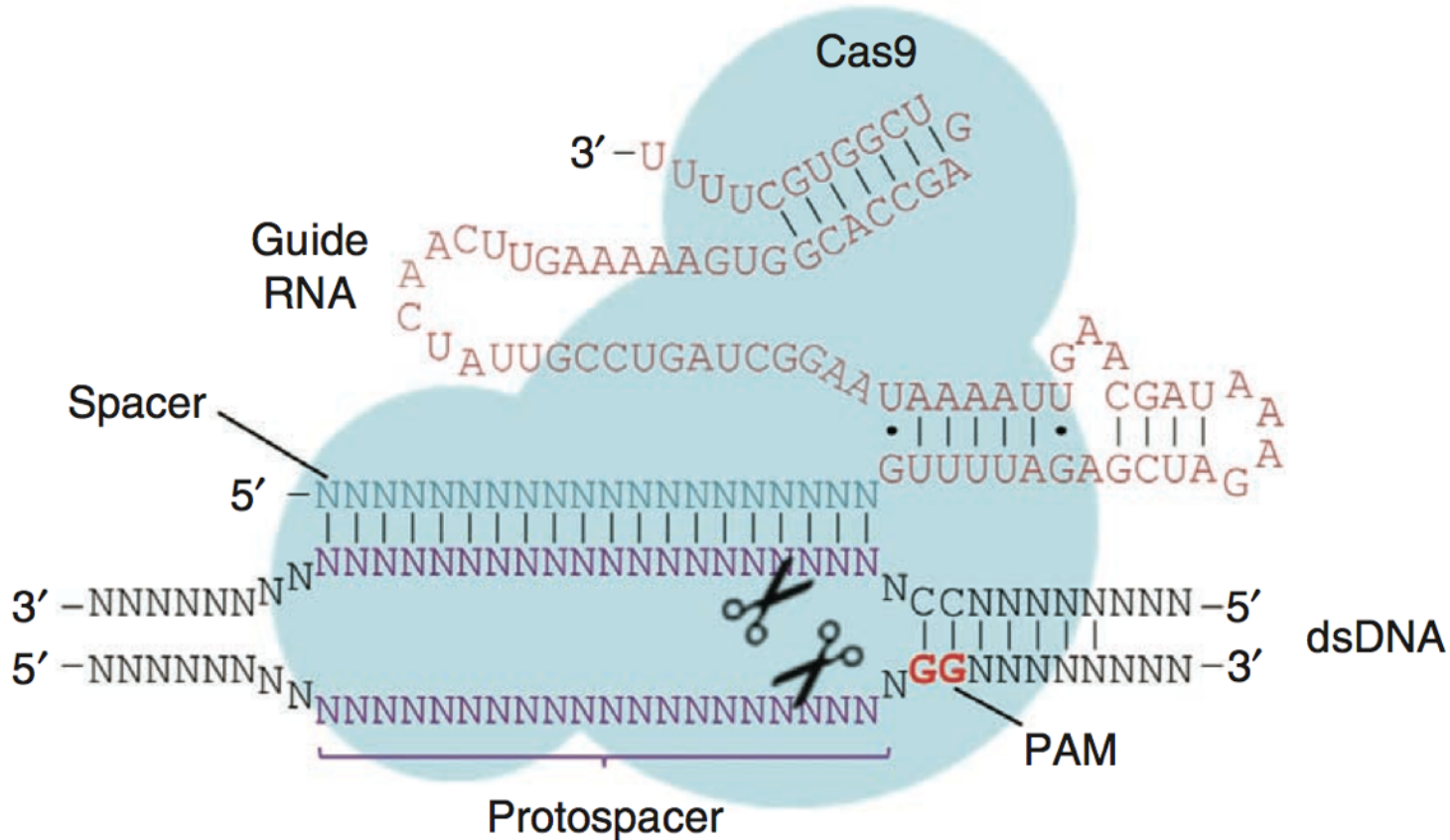


CRISPR-Cas - basics: immunity

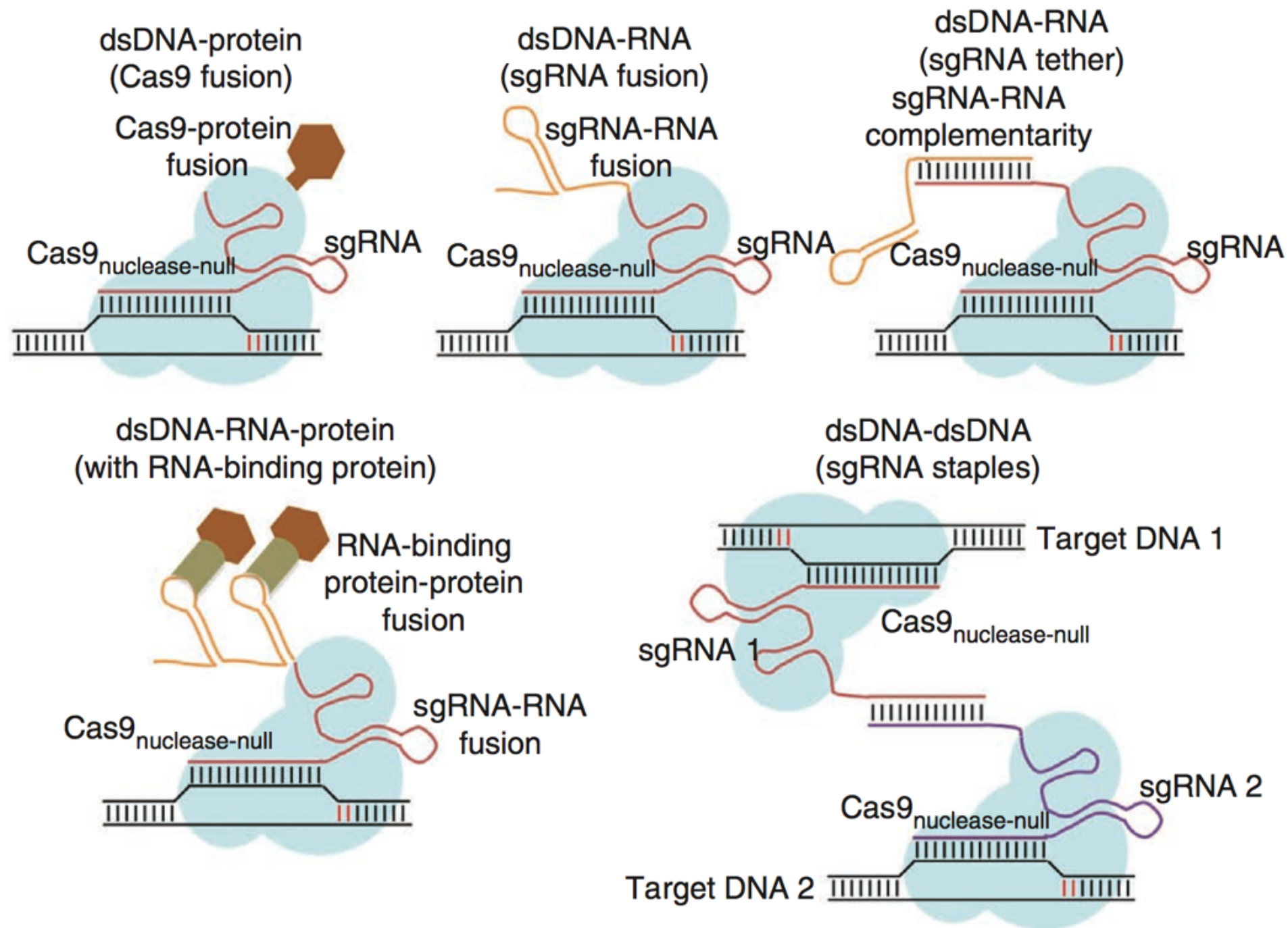
Phase 2: immunity



CRISPR-Cas - targeted application using a Guide RNA (gRNA)



CRISPR - basics and potential



CRISPR - potential applications

Cas9 nuclease or nickase cleavage

Cuts



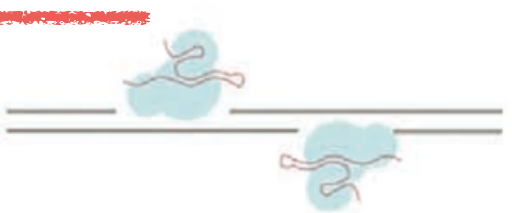
Deletions



Nicks



Offset nicks



Cas9_{nuclease-null} protein fusions

Regulation



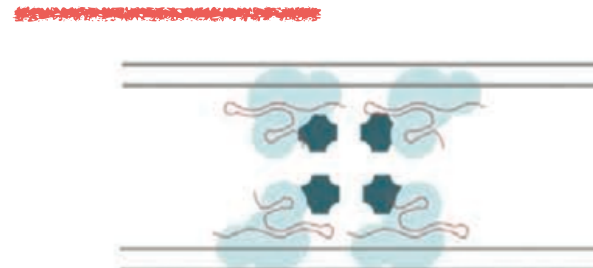
Labeling



Nucleases



Recombinases



Cas9_{nuclease-null} nucleic acid tethers

Regulation



Scaffold



Competition



Aggregation





CRISPR-Cas genome-wide libraries

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Ophir Shalem,^{1,2*} Neville E. Sanjana,^{1,2*} Ella Hartenian,¹ Xi Shi,^{1,3}
David A. Scott,^{1,2} Tarjei S. Mikkelsen,¹ Dirk Heckl,⁴ Benjamin L. Ebert,⁴ David E. Root,¹
John G. Doench,¹ Feng Zhang^{1,2†}

Science 2014

Resource

Cell

Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation

Luke A. Gilbert,^{1,2,3,4,8} Max A. Horlbeck,^{1,2,3,4,8} Britt Adamson,^{1,2,3,4} Jacqueline E. Villalta,^{1,2,3,4}
Yuwen Chen,^{1,2,3,4} Evan H. Whitehead,^{1,3,5,10} Carla Guimaraes,⁶ Barbara Panning,⁷ Hidde L. Ploegh,⁶
Michael C. Bassik,^{1,2,3,4,9} Lei S. Qi,^{1,3,5,10} Martin Kampmann,^{1,2,3,4,*} and Jonathan S. Weissman^{1,2,3,4,*}

Cell 2014



USDA will not regulate gene-edited CRISPR mushroom that resists browning

Emily Waltz | April 15, 2016 | Nature



PRINTER FRIENDLY 

The GLP aggregated and excerpted this blog/article to reflect the diversity of news, opinion and analysis.

The US Department of Agriculture (USDA) will not regulate a mushroom genetically modified with the gene-editing tool CRISPR–Cas9.

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.



White button mushrooms, Photo by Darkone

1 of 6 polyphenol oxidase deleted by CRISPR CAS



Concepts

Restriction:

- site-specific cleavage of palindromic DNA by restriction enzymes

Assembly:

- isothermal fusion of dsDNA fragments using exonucleases, polymerases, and ligases

Plasmids:

- mobile genetic elements used for cloning and expression of genes

Homologous Recombination:

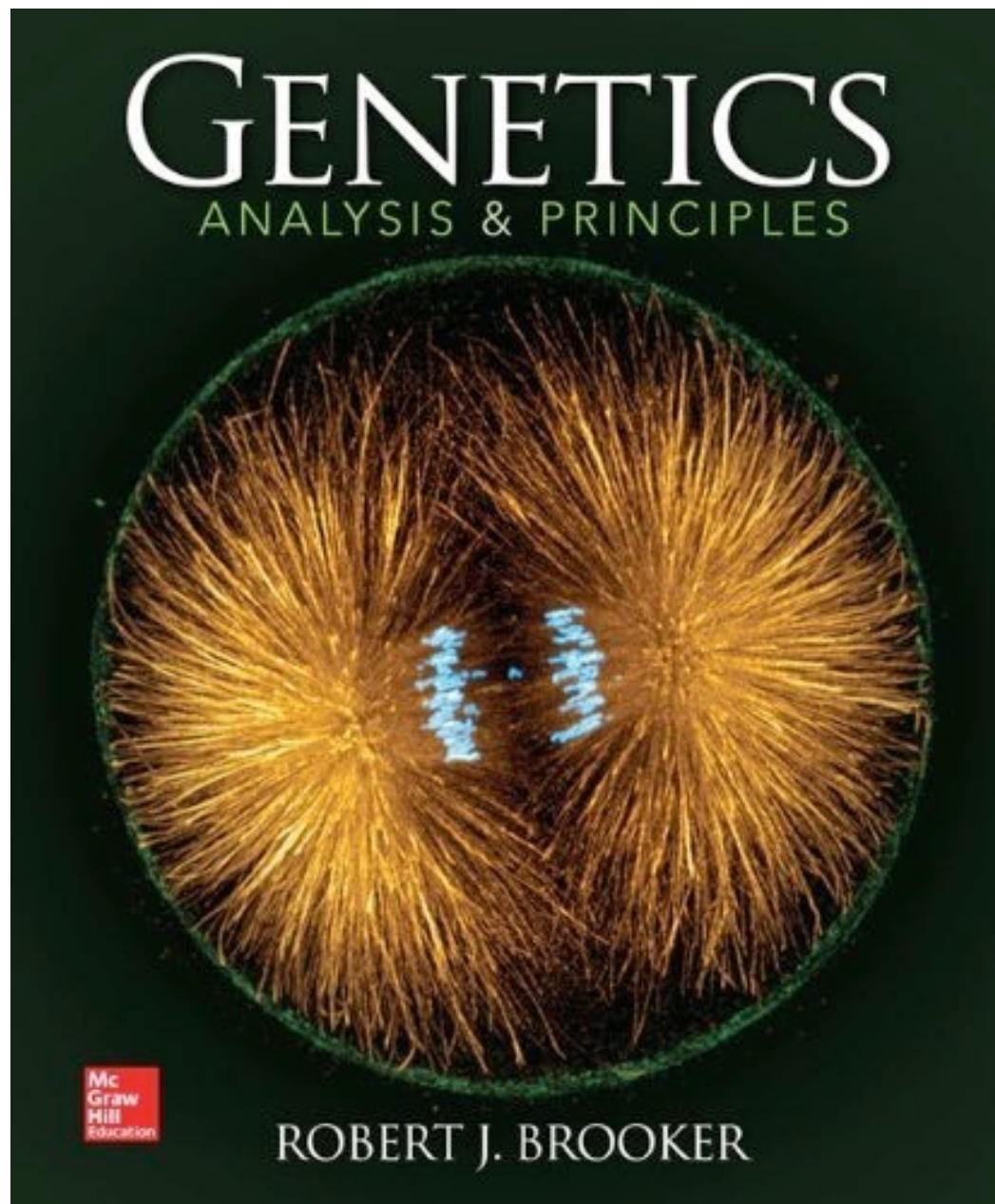
- cross-over (exchange) of homologues DNA fragments

Site-specific DNA editing: requires double strand breaks

- protein-guided nucleases: ZFN, TALEN
 - RNA-guided nucleases: CRISPR-Cas
-



Resources



5th edition 2014

- Gaj, T., Gersbach, C. A., & Barbas, C. F. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*. <http://doi.org/10.1016/j.tibtech.2013.04.004>
- Mali, P., Esvelt, K. M., & Church, G. M. (2013). Cas9 as a versatile tool for engineering biology. *Nature Methods*, 10(10), 957–963. <http://doi.org/10.1038/nmeth.2649>
- Sander, J. D., & Joung, J. K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*, 32(4), 347–355. <http://doi.org/10.1038/nbt.2842>



Questions?



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E.-Aulhorn-Str. 6, Room 1.1.05
