https://www.nature.com/news/crispr-everywhere-1.19511

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Joel Sharbrough

# Clustered Regularly Interspaced Palindromic Repeats (CRISPRs)

"A characteristic of the CRISPRs, not seen in any other class of repetitive DNA, is that the repeats of the CRISPRs are interspaced by similarly sized non-repetitive DNA. The direct repeats varies in size from 21 bp in *Salmonella typhimurium* to 37 bp in *Streptococcus pyogenes*, and they are clustered in one or several loci on the chromosome."

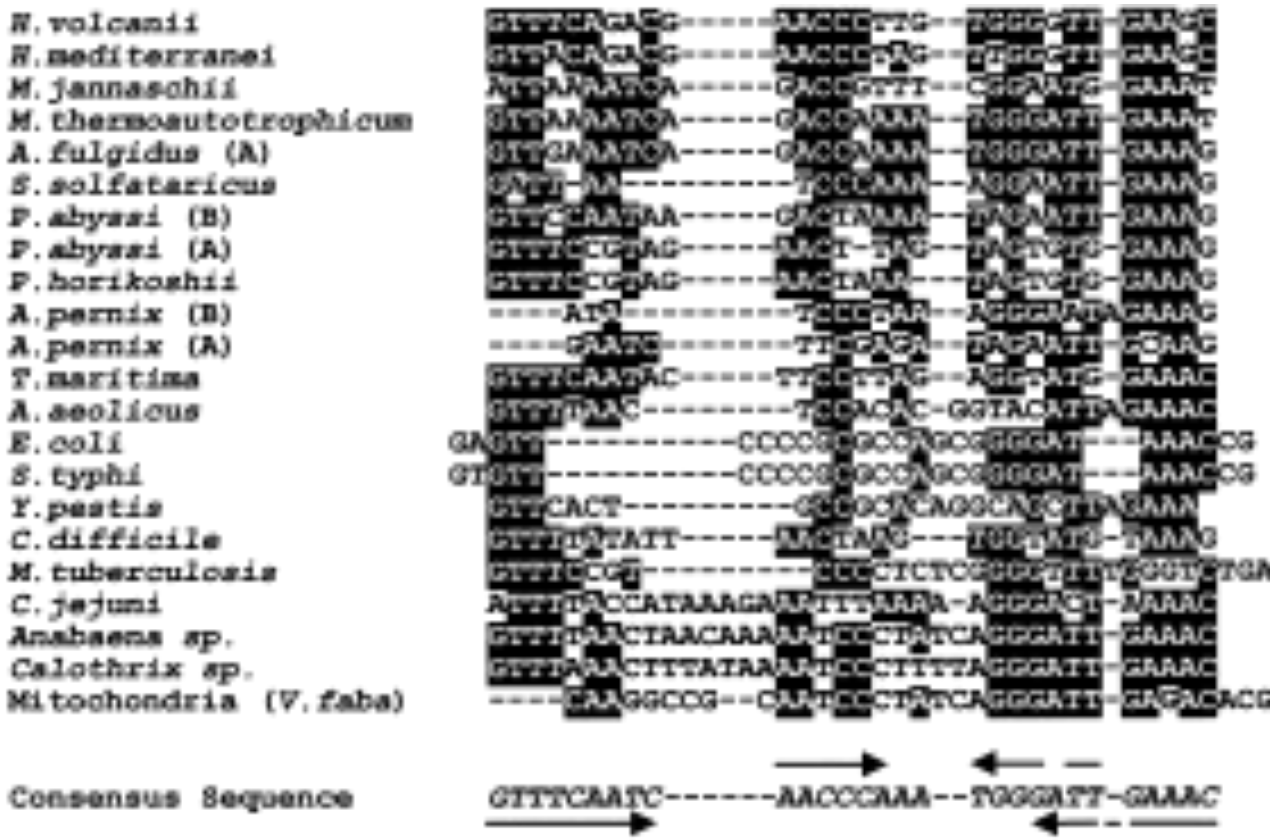
Jansen et al., 2002. Mole. Microbiol. 43(6), 1565–1575.

# CRISPRs are ancient and highly conserved

**Table 1.** Main features of the SRSRs.

| Organism | SRSR size (bp) | Spacing (bp) | Number of clusters | SRSR units per cluster | Reference |
|---|---|---|---|---|---|
| **Archaea** | | | | | |
| *H. volcanii* | 30 | ND | ≥2 | ND | Mojica *et al.* (1995) *M* |
| *H. mediterranei* | 30 | 33–39 | 3 | 21/ ND / ND | Mojica *et al.* (1995) *M* |
| *M. jannaschii* | 28–30 | 31–51 | $7^A + 6^B + 1^C$ | 4–25 | Bult *et al.* (1996) *Scie* |
| *M. thermoautotrophicum* | 30 | 34–38 | 2 | 124/47 | This work |
| *A. fulgidus* | $37^A/30^B$ | ≈ 37 | $1^A + 2^B$ | $42^A/48^B/60^B$ | This work |
| *S. solfataricus* | 25 | ≈ 40 | ≥2 | 94/102 | Sensen *et al.* (1998) |
| *P. abysii* | $29^A/30^B$ | 26–43 | $1^A + 2^B$ | $7^A/22^B/27^B$ | This work |
| *P. horikoshii* | 29 | 34–58 | 3 | 18/26/66 | Kawarabayasi *et al.* ( |
| *A. pernix* | $24^A/23^B$ | 37–52 | $2^A + 1^B$ | $19^A/27^A/42^B$ | Kawarabayasi *et al.* ( |
| **Bacteria** | | | | | |
| *T. maritima* | 30 | 39–40 | 8 | 2–40 | Nelson *et al.* (1999) *M* |
| *A. aeolicus* | 29 | 36–38 | 1 | 6 | This work |
| *E. coli* | 29 | 32–33 | 3 | 2/7/13 | Nakata *et al.* (1989) *J* |
| *S. typhi* | 29 | 32 | ≥1 | 6 | This work |
| *C. jejuni* | 36 | 30 | 1 | 5 | This work |
| *Y. pestis* | 28 | 32–33 | 2 | 6/9 | This work |
| *C. difficile* | 29 | 36–38 | $4^A + 2^B$ | 5–17 | This work |
| *M. tuberculosis* | 36 | 38–40 | 1 | Variable | Hermans *et al.* (1991 |
| *Calothrix sp.* | 37 | 35–41 | >1 | 5 | Masepohl *et al.* (1996 |
| *Anabaena sp.* | 37 | 32–43 | >1 | 17 | Masepohl *et al.* (1996 |
| **Mitochondria** | | | | | |
| *V. faba* | 40 | 20–35 | 1 | 6 | Flamand *et al.* (1992) |

A,B, Types of SRSRs distinct (more than 3 bp differences) within the same microorganism. ND, Not d




Mojica et al., 2000. Mole. Microbiol. 36(1), 244-246.

# First observation of a CRISPR-like element in 1987

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0

Vol. 169, No. 12

## Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

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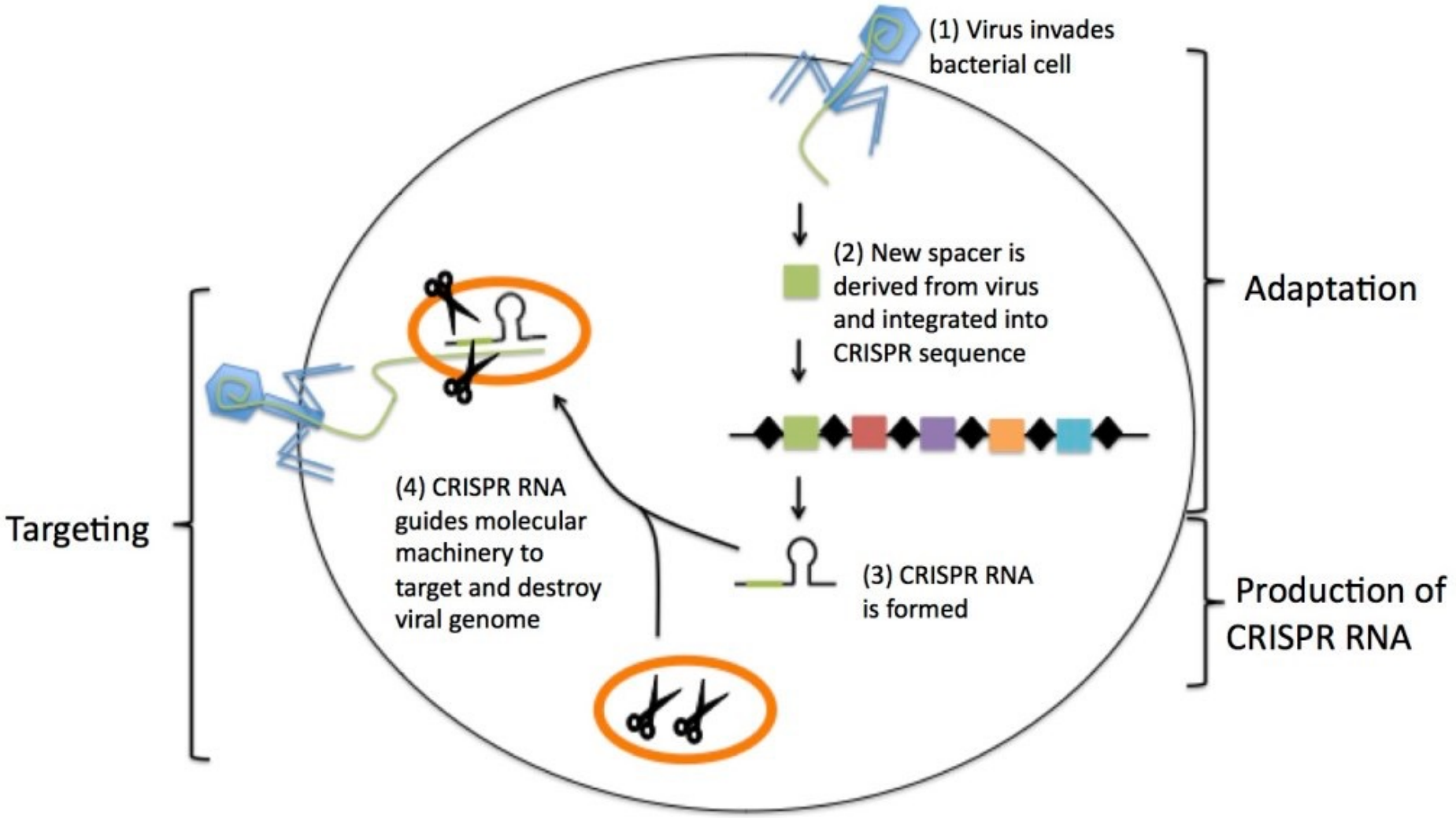
Received 1 May 1987/Accepted 22 August 1987

**The *iap* gene in *Escherichia coli* is responsible for the isozyme conversion of alkaline phosphatase. We analyzed the 1,664-nucleotide sequence of a chromosomal DNA segment that contained the *iap* gene and its flanking regions. The predicted *iap* product contained 345 amino acids with an estimated molecular weight of 37,919. The 24-amino-acid sequence at the amino terminus showed features characteristic of a signal peptide. Two proteins of different sizes were identified by the maxicell method, one corresponding to the Iap protein and the other corresponding to the processed product without the signal peptide. Neither the isozyme-converting activity nor labeled Iap proteins were detected in the osmotic-shock fluid of cells carrying a multicopy *iap* plasmid. The Iap protein seems to be associated with the membrane.**

# How do CRISPRs provide DNA-encoded, RNA-mediated, sequence-specific immunity?

# CRISPR-Cas9 and the bacterial immune system

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
CRISPR ASsociated Endonuclease 9 (Cas9)

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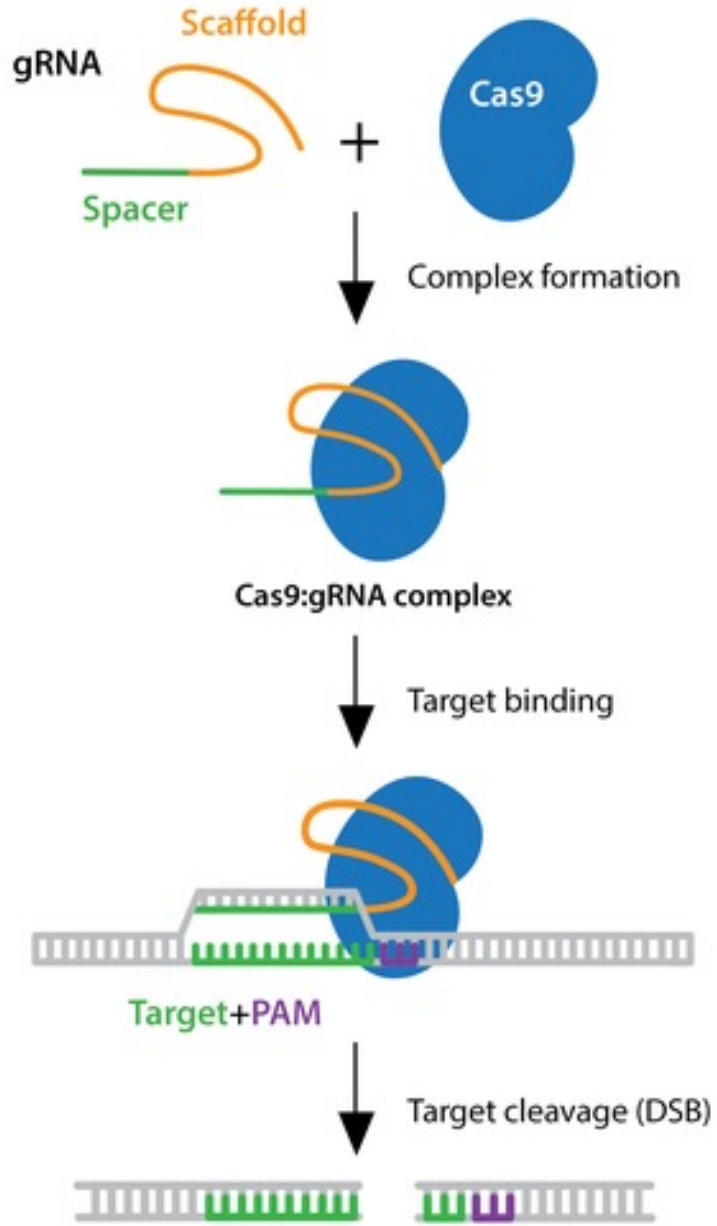
# CRIPSR basics




CRISPR RNA = Guide RNA (gRNA) = Spacer + Scaffold

Spacer: a unique sequence (~20bp) that targets Cas9 to a specific area of the genome

Scaffold: a repetitive sequence (~70bp) required for Cas9 binding and activity

Protospacer Adjacent Motif (PAM) – generally 3-6bp long (NGG), always read in the 5' -> 3' direction. Required for Cas9 recognition and endonuclease activity

Double Strand Break (DSB) – DNA cleavage caused by the two nuclease domains of Cas9

Addgene.org

Slide by Amanda Broz


# Using CRISPR/Cas9 to edit genomes


# Using CRISPR/Cas9 to edit genomes

# Use of fused tracrRNA:crRNA to guide Cas9 to cleave specific DNA target

## 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) systems 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 a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

Jinek et al., 2012. Scien

# Patent Wars: UC Berkeley vs. Broad Institute

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2012 Prokaryotes
UC Berkeley

vs.

## 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.

2013 Eukaryotes
Broad

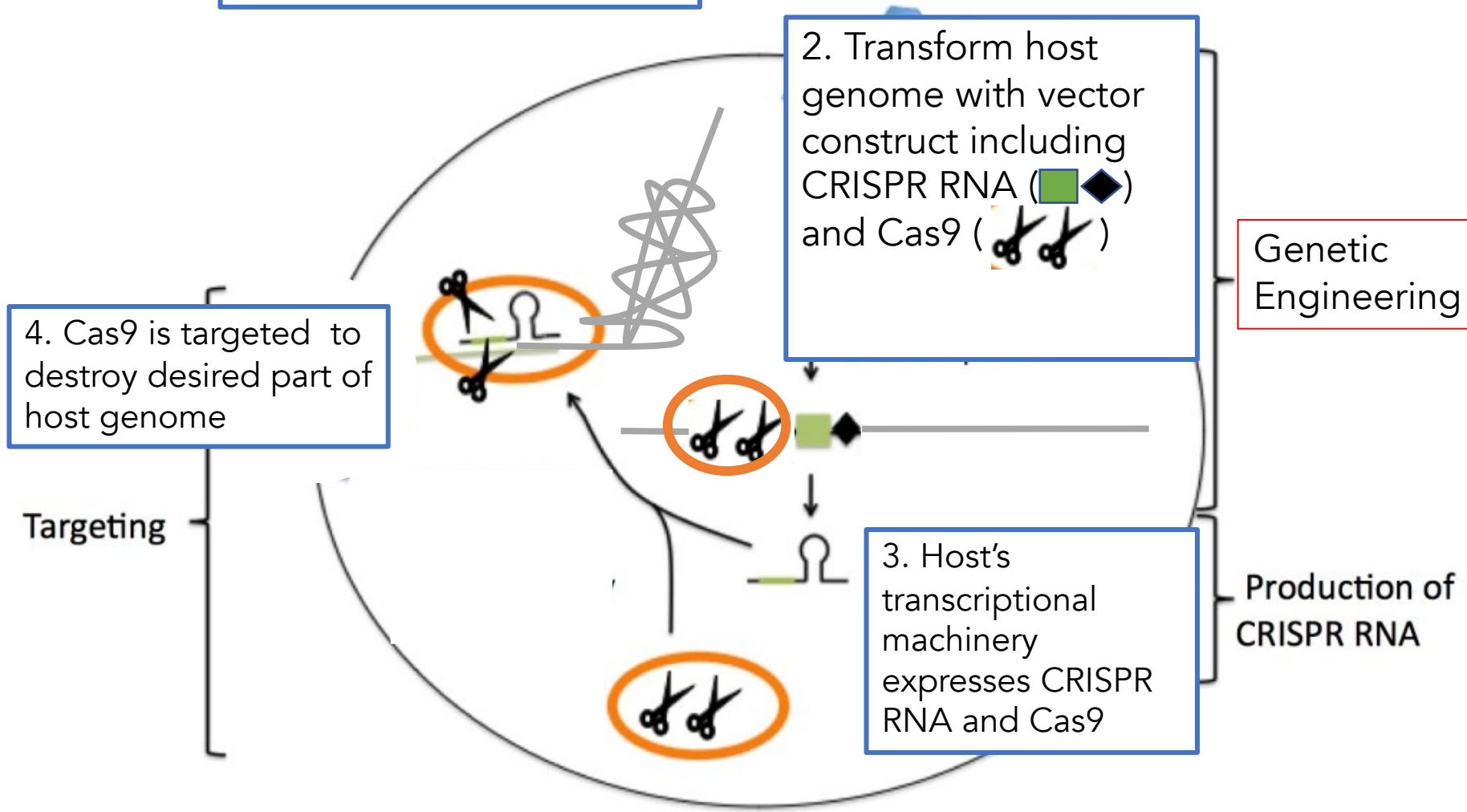
# How can CRISPR/Cas9 be used to make specific genetic edits?

# Using methods from the bacterial immune system to edit genomes

1. Scientist designs a unique CRISPR RNA ( ■ ) to target gene of interest

2. Transform host genome with vector construct including CRISPR RNA (■◆) and Cas9 ( ✂ )

Genetic Engineering



4. Cas9 is targeted to destroy desired part of host genome

Targeting

3. Host's transcriptional machinery expresses CRISPR RNA and Cas9

Production of CRISPR RNA

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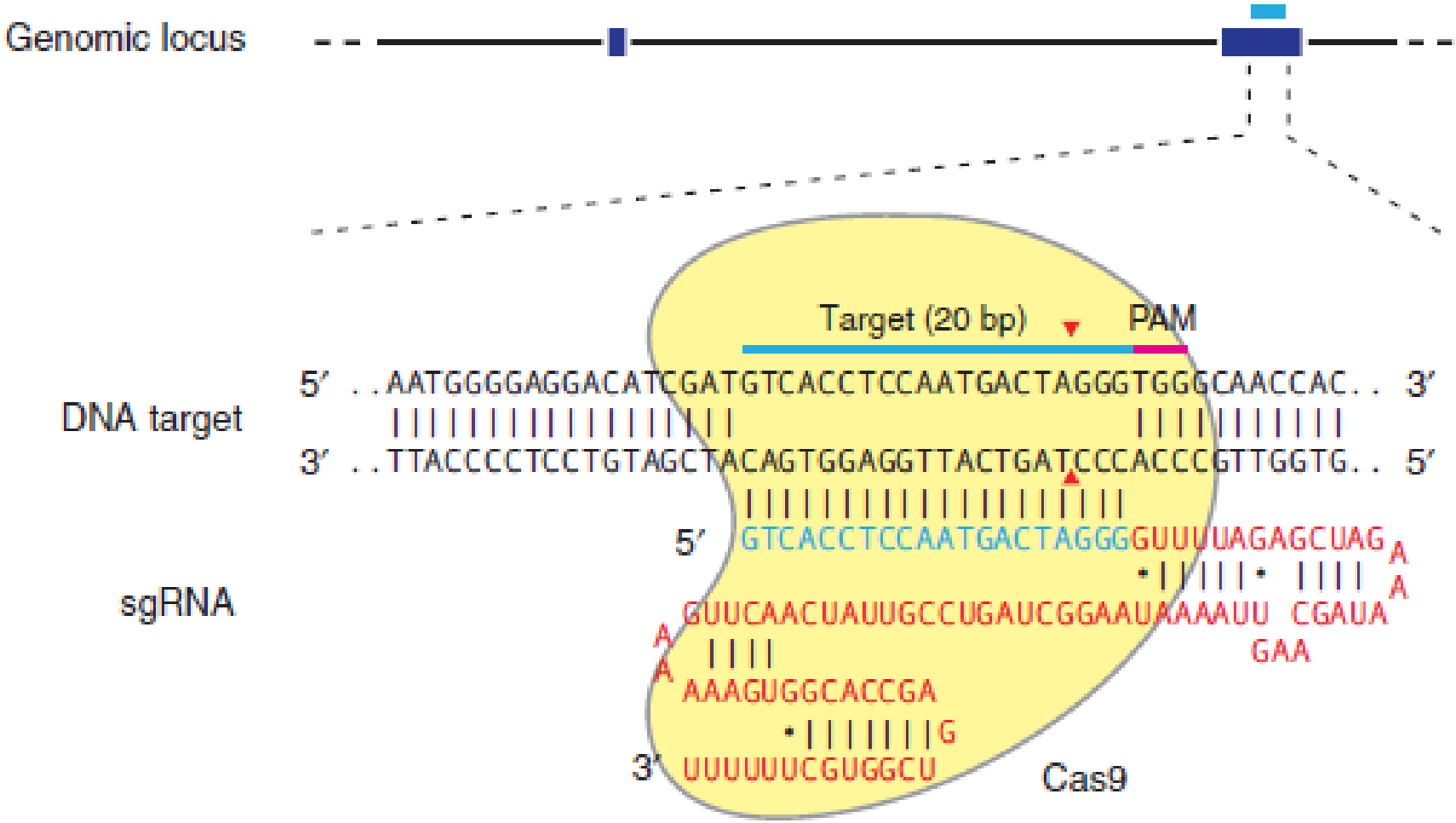
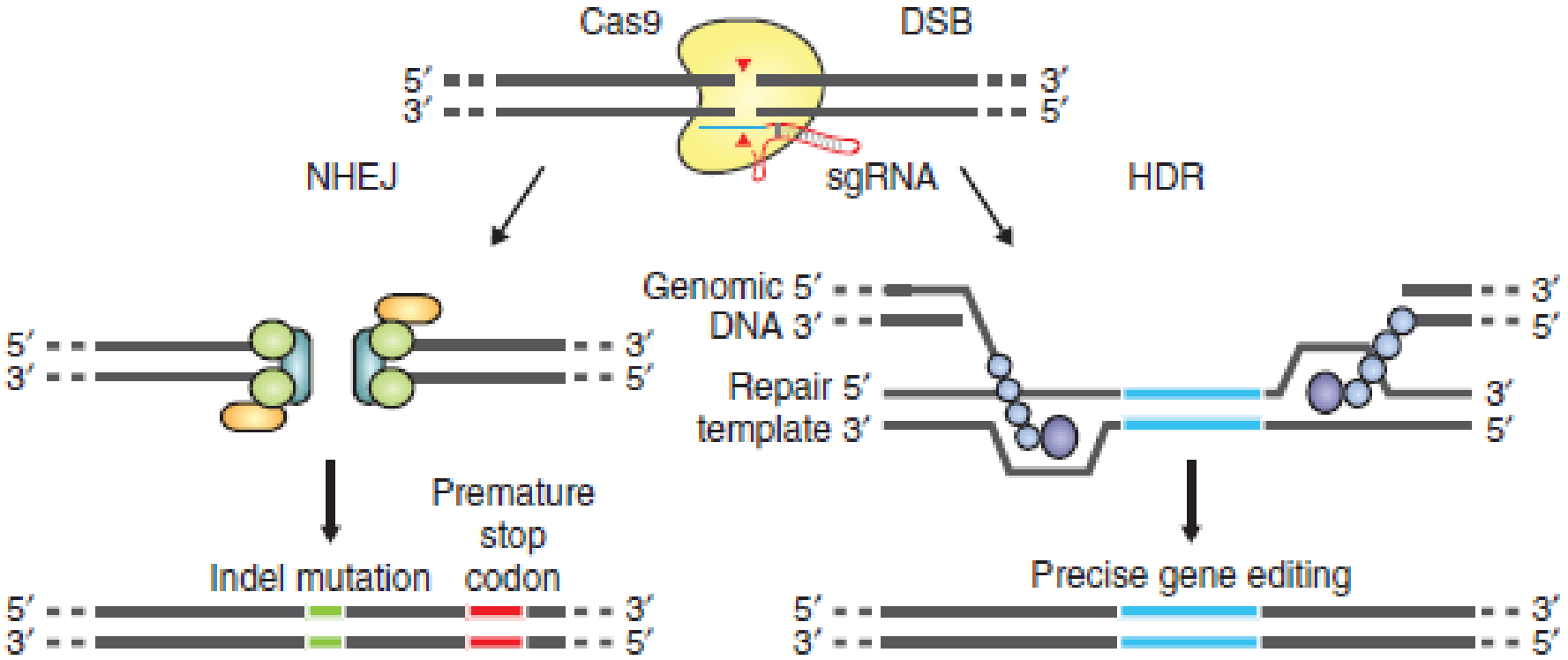
# Close up of gRNA targeting and DNA cleavage site




Slide by Amanda Broz

Ran et al. 2013

# Double strand break repair – What type of mutation will result from gene editing?



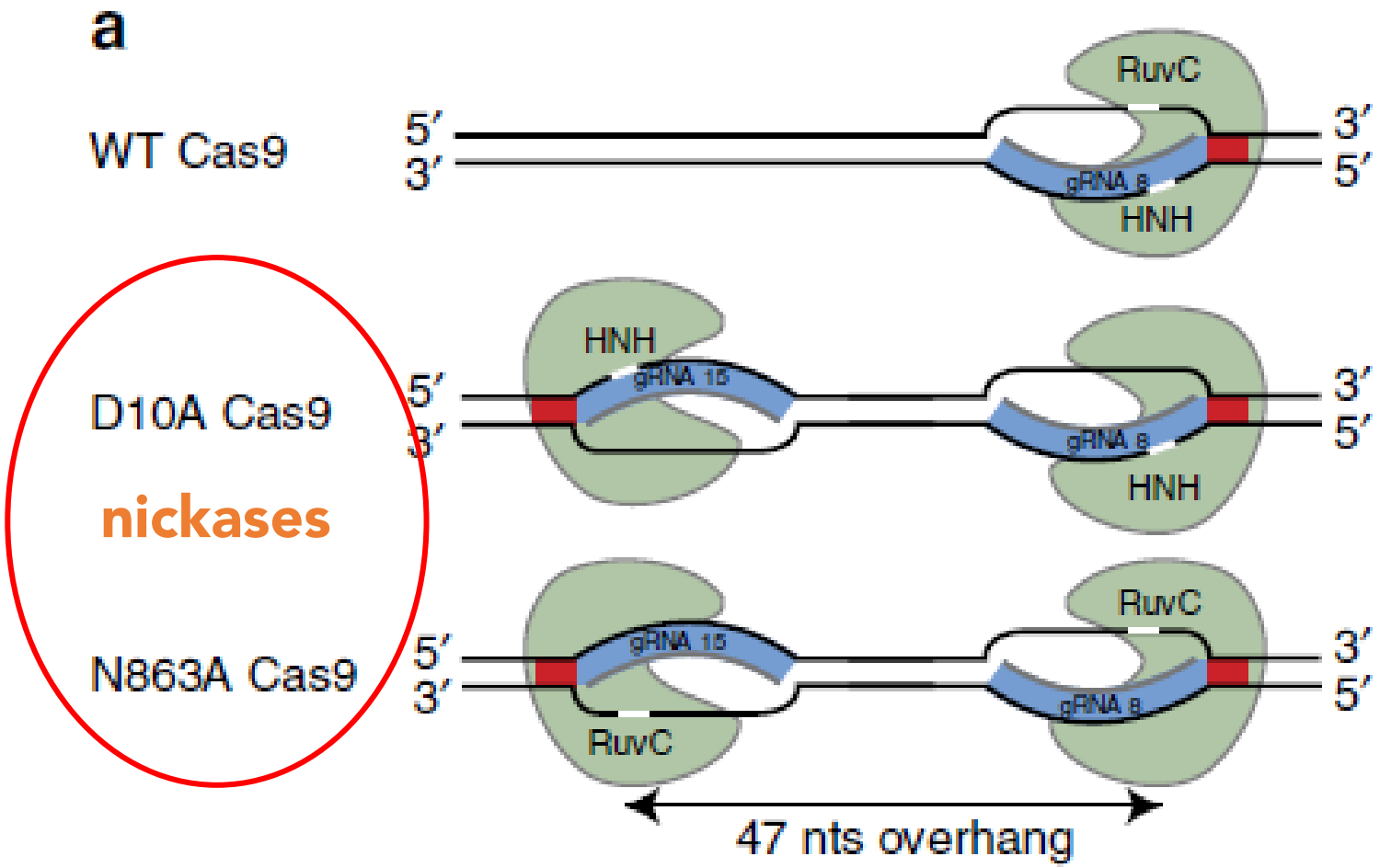

Non-homologous End Joining (NHEJ)
Knock out/mutate your gene

Homology Directed Repair (HDR)
Replaces original DNA sequence with sequence of your choice using exogenous 'donor template' (low efficiency).

Slide by Amanda Broz

Ran et al. 2013

# Off-targets can be greatly reduced (to almost zero) using paired gRNAs and a Cas9 'nickase'




Single nicks are efficiently repaired; only double nicks result in mutations

**Extra bonus** – nickases generally create large deletions in DNA

Slide by Amanda Broz

Bothmer et al. 2017

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- Does the short length of the PAM recognition sequence promote immune breadth?
- Are there analogs to CRISPR found in the wild that **have been or might eventually b**e co-opted for use in molecular biology?