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About us

GENE EDITING AND EMBRYOLOGY FACILITY

Based at the EMBL Outstation in ROME, Monterotondo

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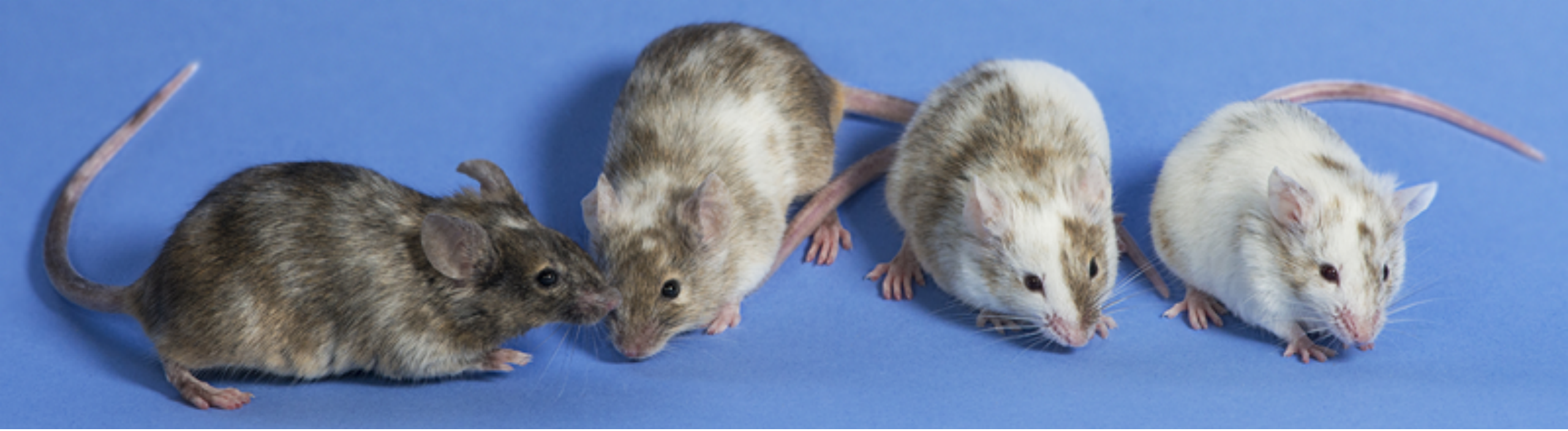

„In biology, as in mechanics, one of the best ways to figure out how something works is to break it.“

Vogel, *Science* (2000) 288, 1160

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Genome editing

- To understand the function of a gene or a protein: direct mutation and study of the effects on the organism.
- To understand relation between genes and diseases thus improving available therapeutic approaches (i.e. cancer, heritable diseases).
- Revealing unknown biological procesesses that could be crucial in the establishment of a defined pathology.

How do we make genetically modified mice?

We genetically manipulate the embryos

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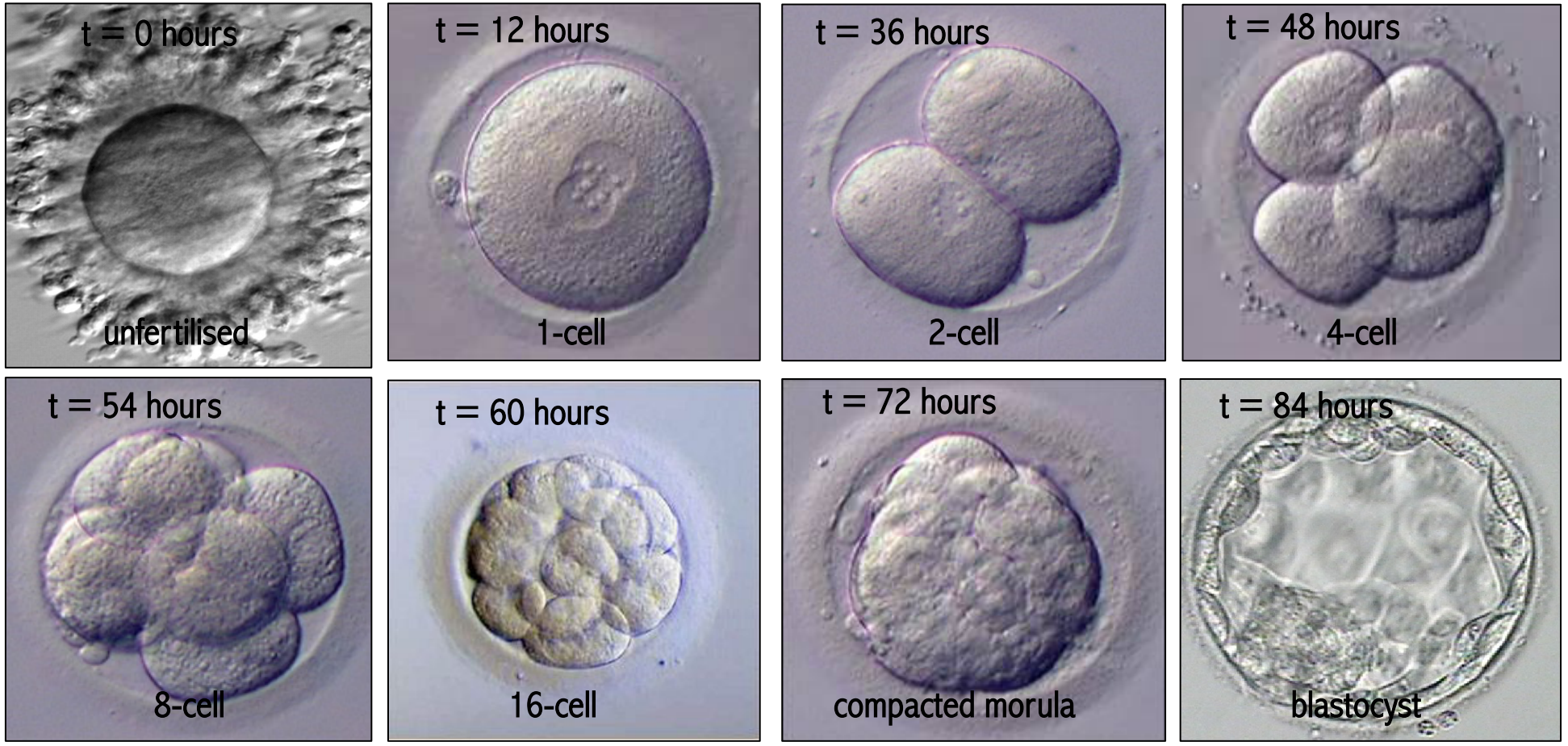
t = 0 hours
unfertilised
t = 12 hours
1-cell
t = 36 hours
2-cell
t = 48 hours
4-cell
t = 54 hours
8-cell
t = 60 hours
16-cell
t = 72 hours
compacted morula
t = 84 hours
blastocyst

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Transgenic mice

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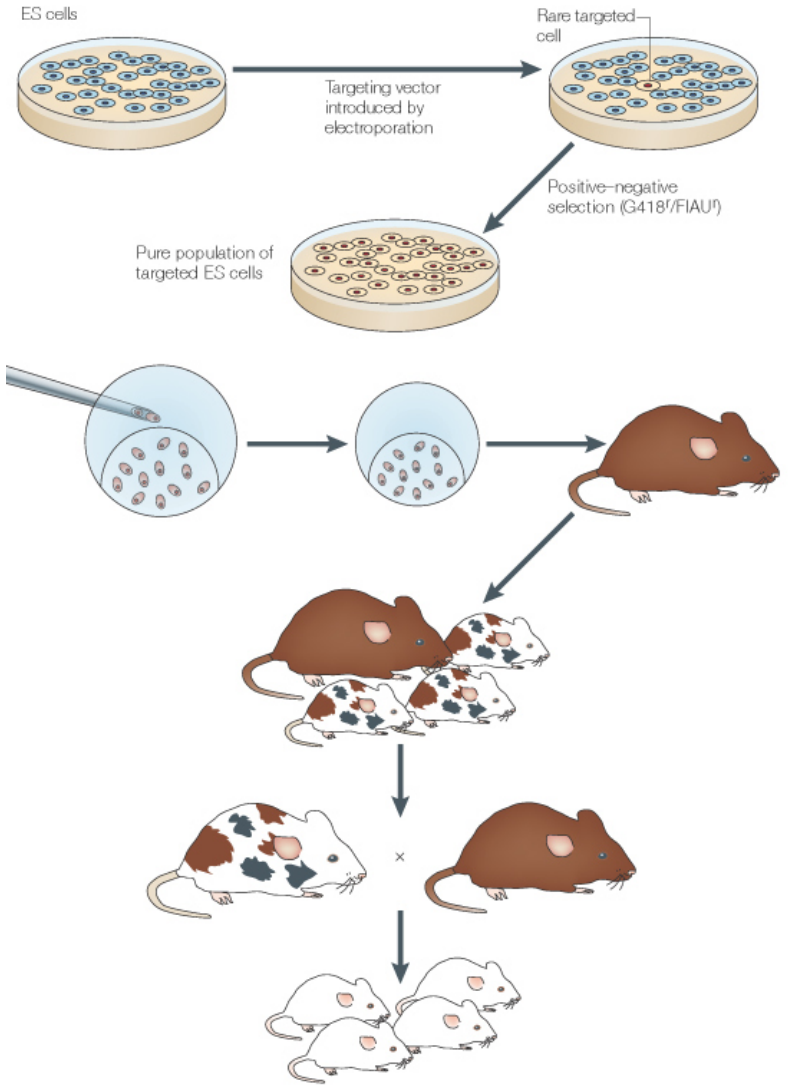
Genetically modified ES cells



Capecchi (2005) *Nature Reviews Genetics 6*, 507

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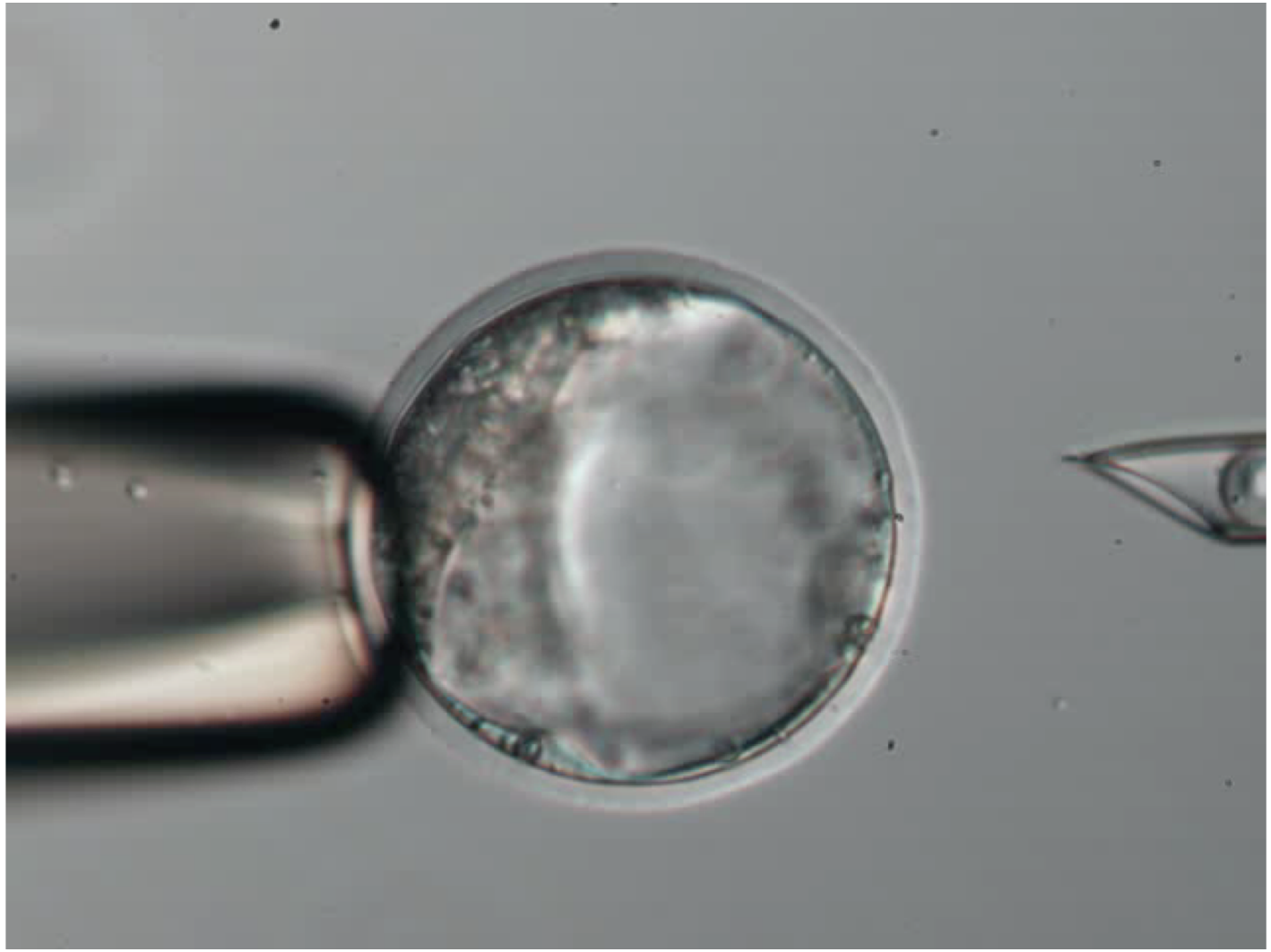
Genetically modified ES cells



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CRISPR-Cas

RESEARCH ARTICLE

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]‡

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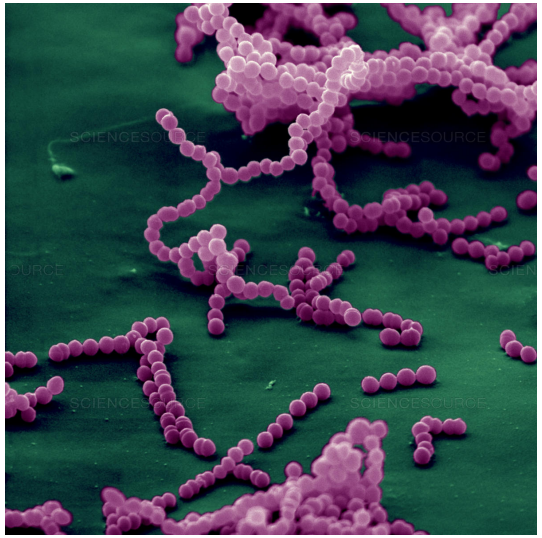
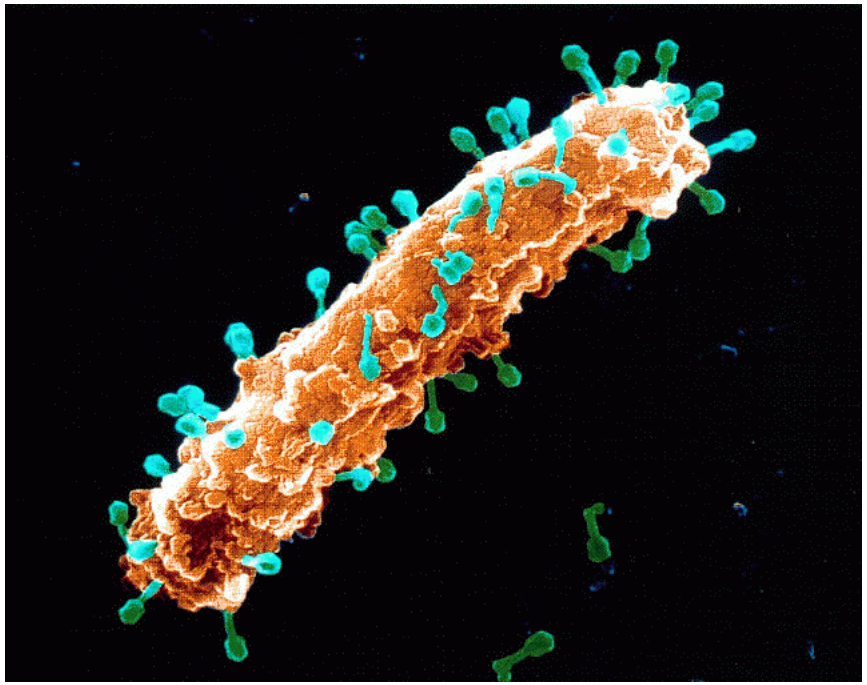
CRISPR-Cas: its simple origin



Streptococcus pyogenes

Viruses



Viruses on bacteria

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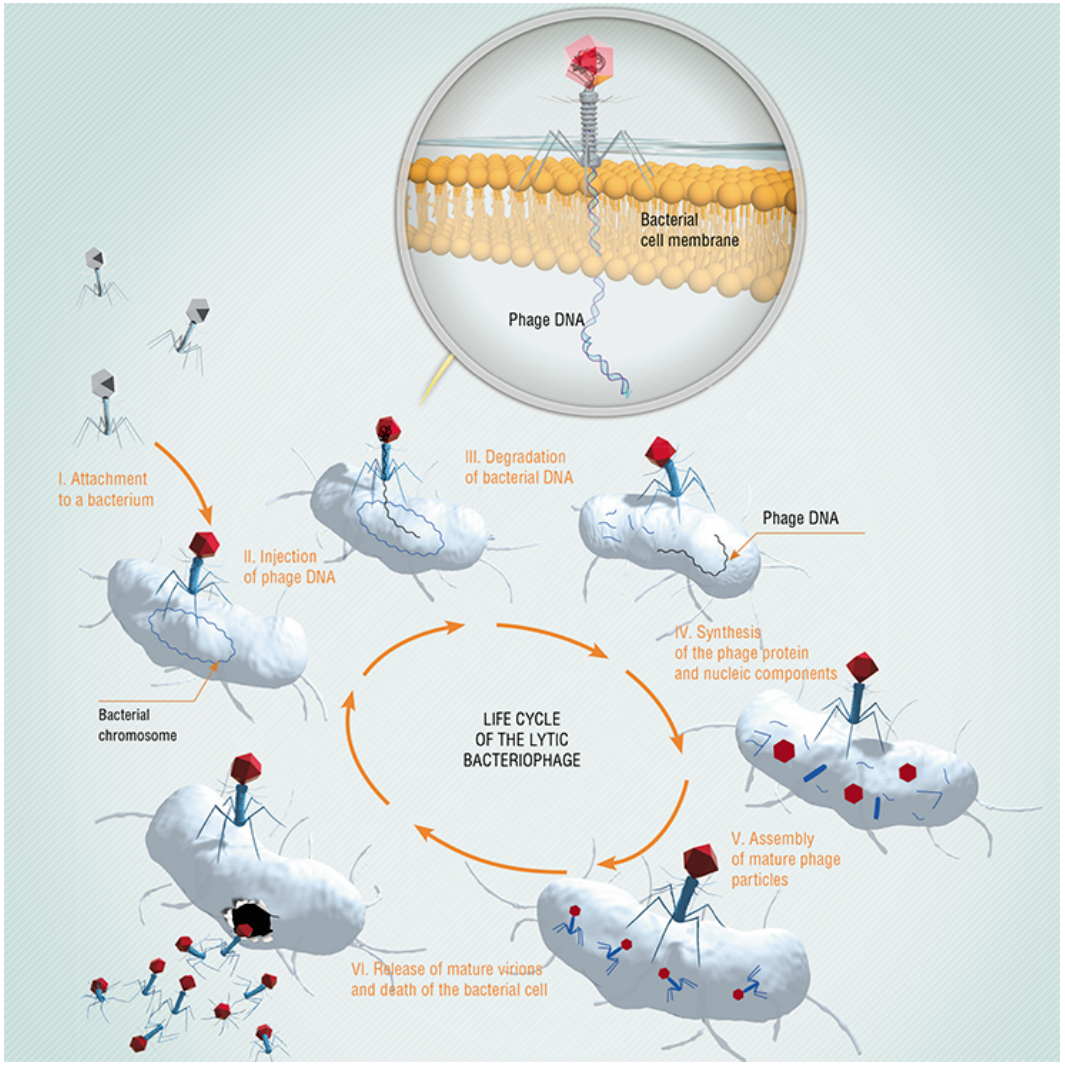
CRISPR-Cas: its simple origin



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Bacteria has evolved against viruses..

- **RESTRICTION ENZYMES**:

DNA-cutting enzymes that recognizes specific sequences.

They act as **molecular scissors** of the viral DNA

- **IMMUNE SYSTEM:**

It is a defense against viruses infection well known in almost 50% of the bacteria, but also present in Archea and Eubacteria.

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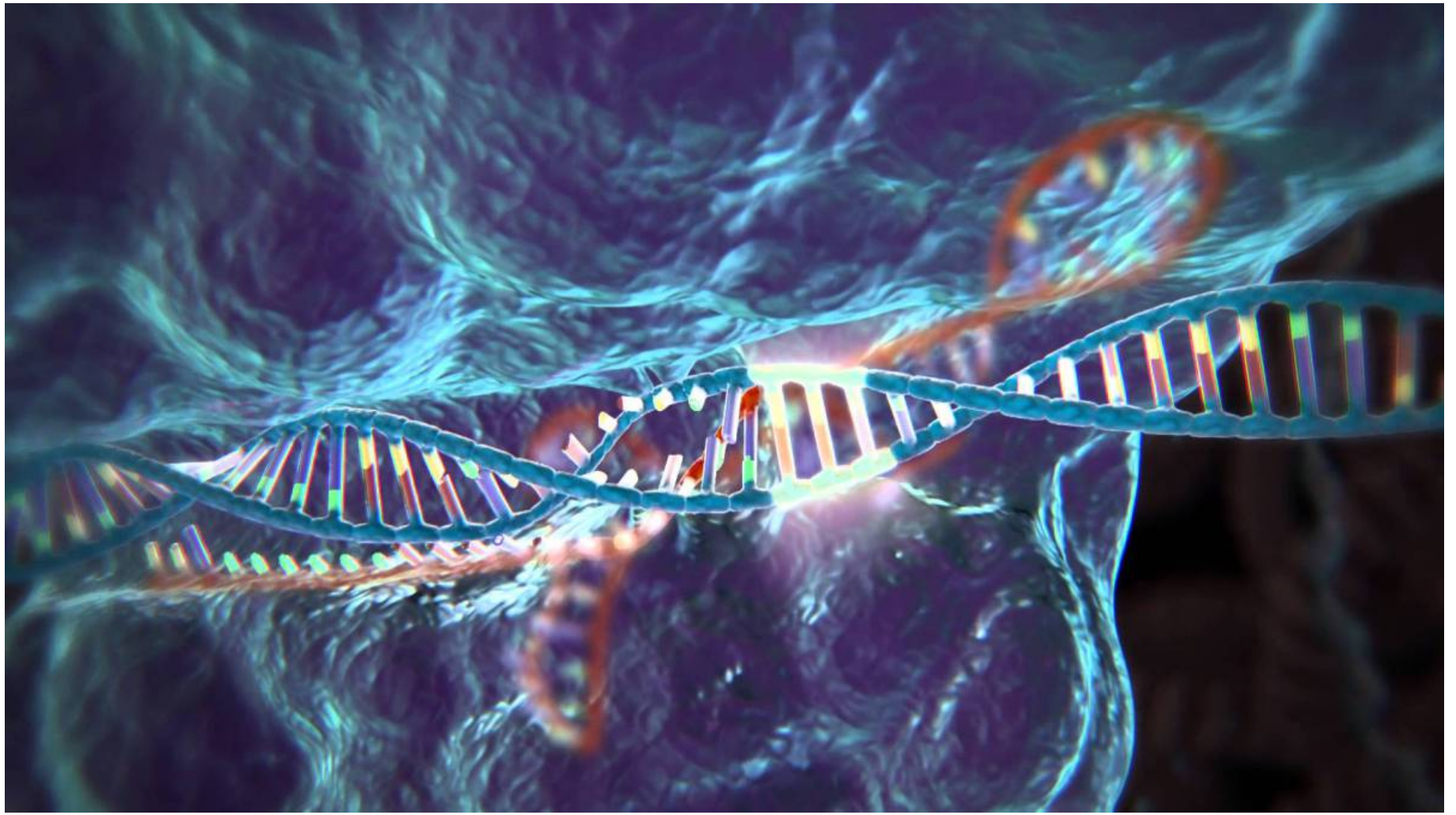
How does CRISPR work in bacteria?



C: Clustered
R: Regularly
I: Interspaced
S: Short
P: Palindromic
R: Repeats

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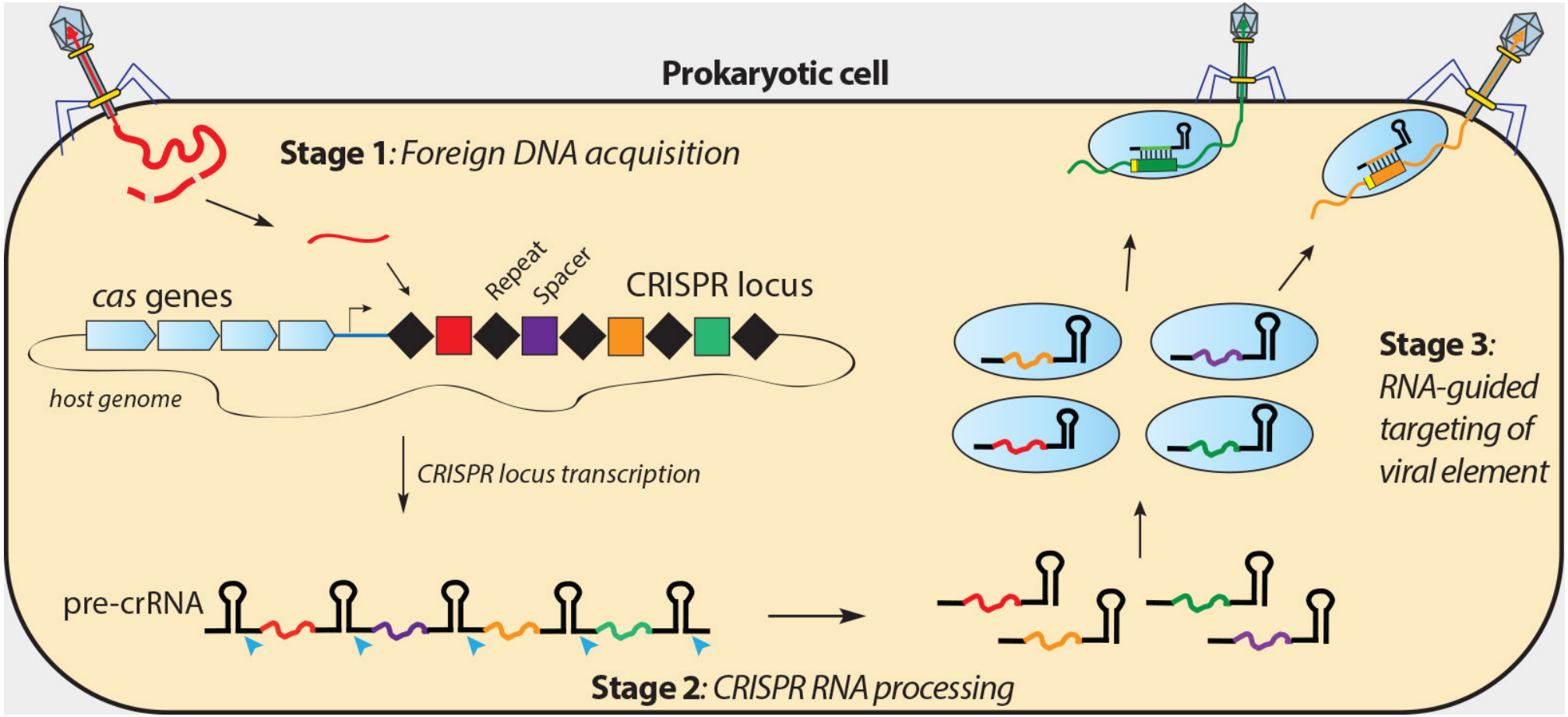
How does CRISPR work in bacteria?



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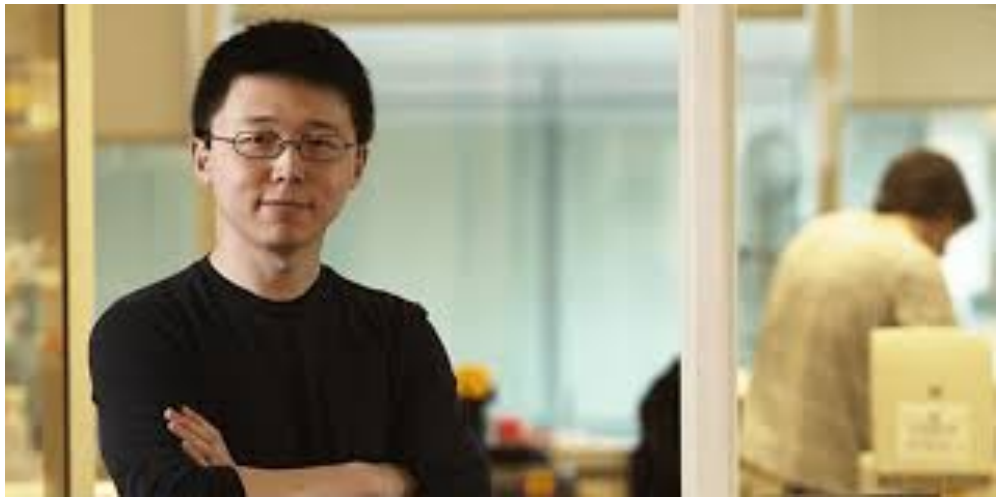

Feng Zhang

PROTOCOL

Genome engineering using the CRISPR-Cas9 system

F Ann Ran[1–5,8], Patrick D Hsu[1–5,8], Jason Wright[1], Vineeta Agarwala[1,6,7], David A Scott[1–4] & Feng Zhang[1–4]

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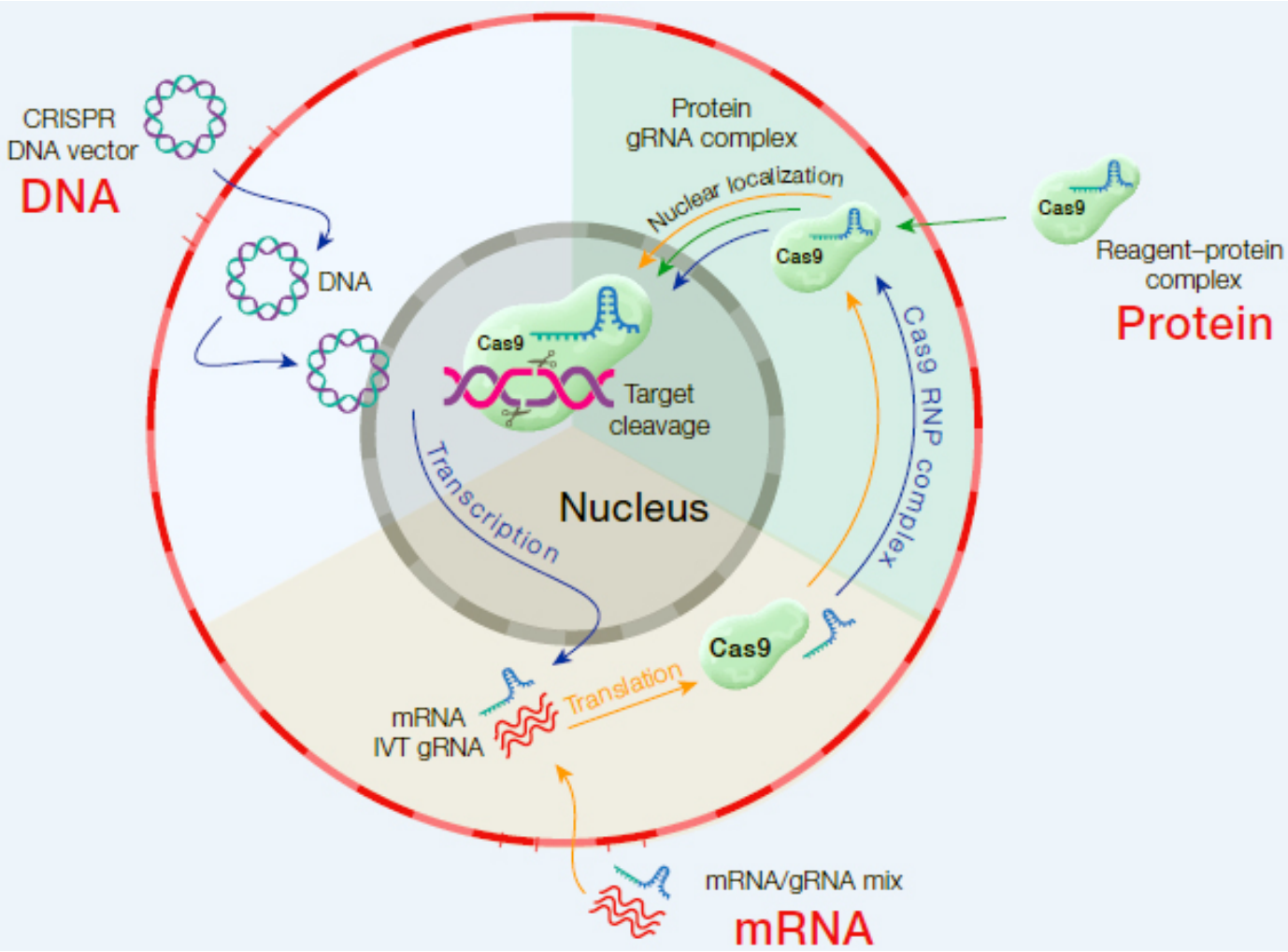
CRISPR-Cas system works also in eukaryotic cells



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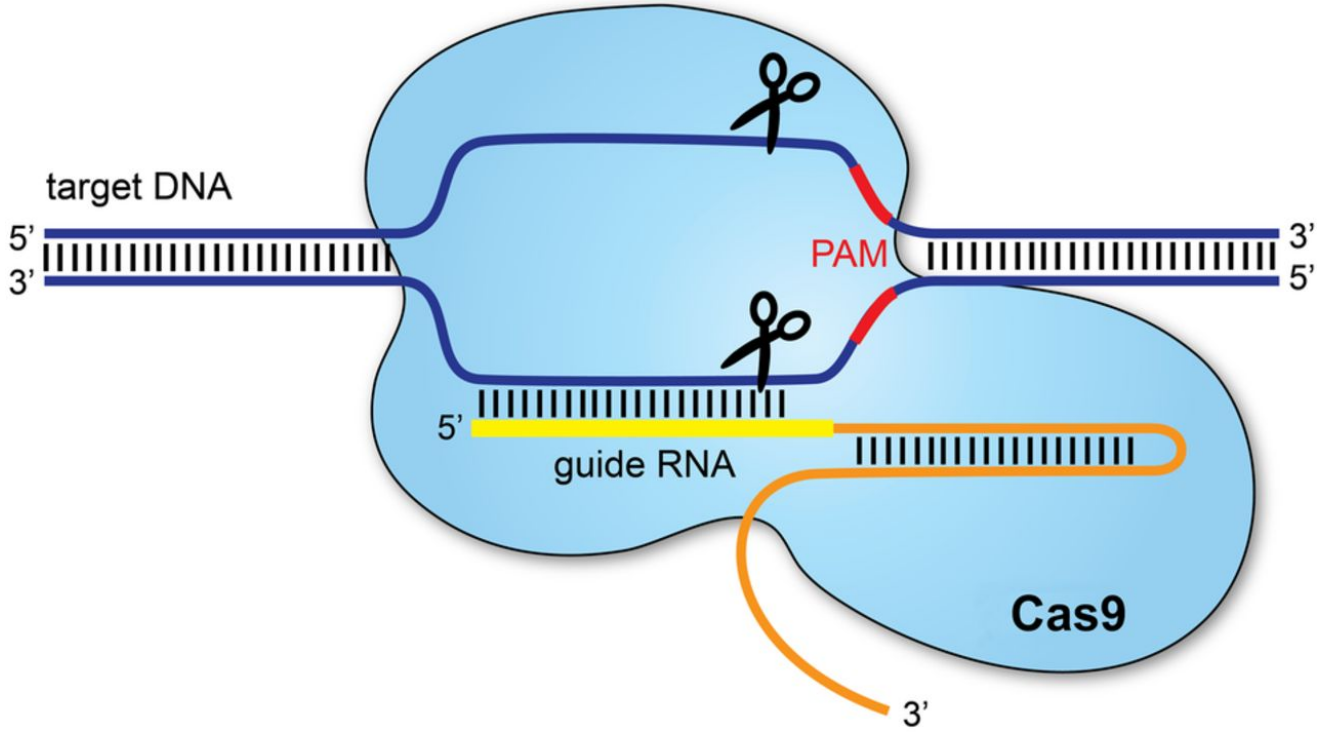
Molecular mechanism of the CRISPR-Cas system



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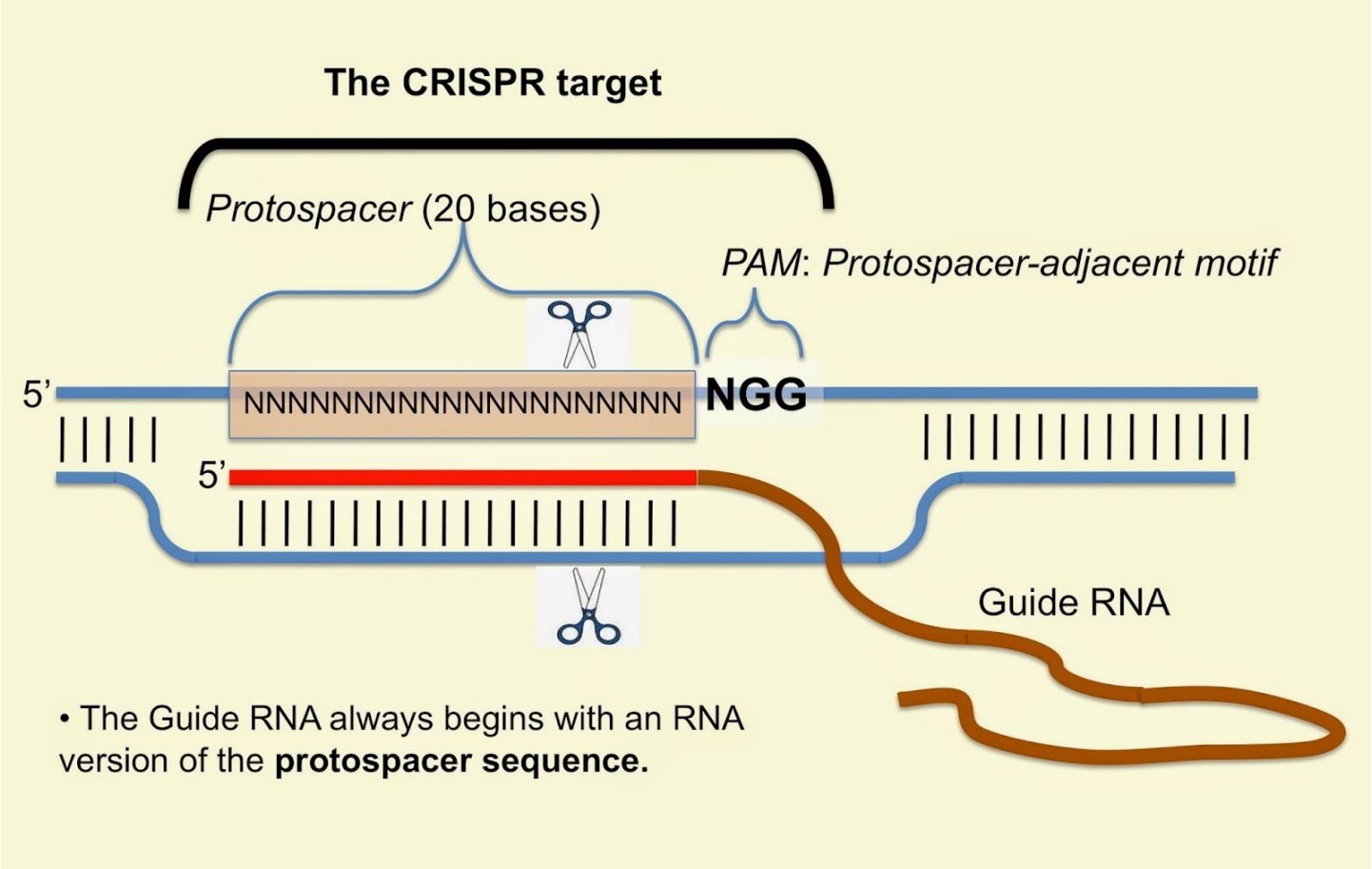
Cas9 can cut any target dsDNA: PAM site is crucial!



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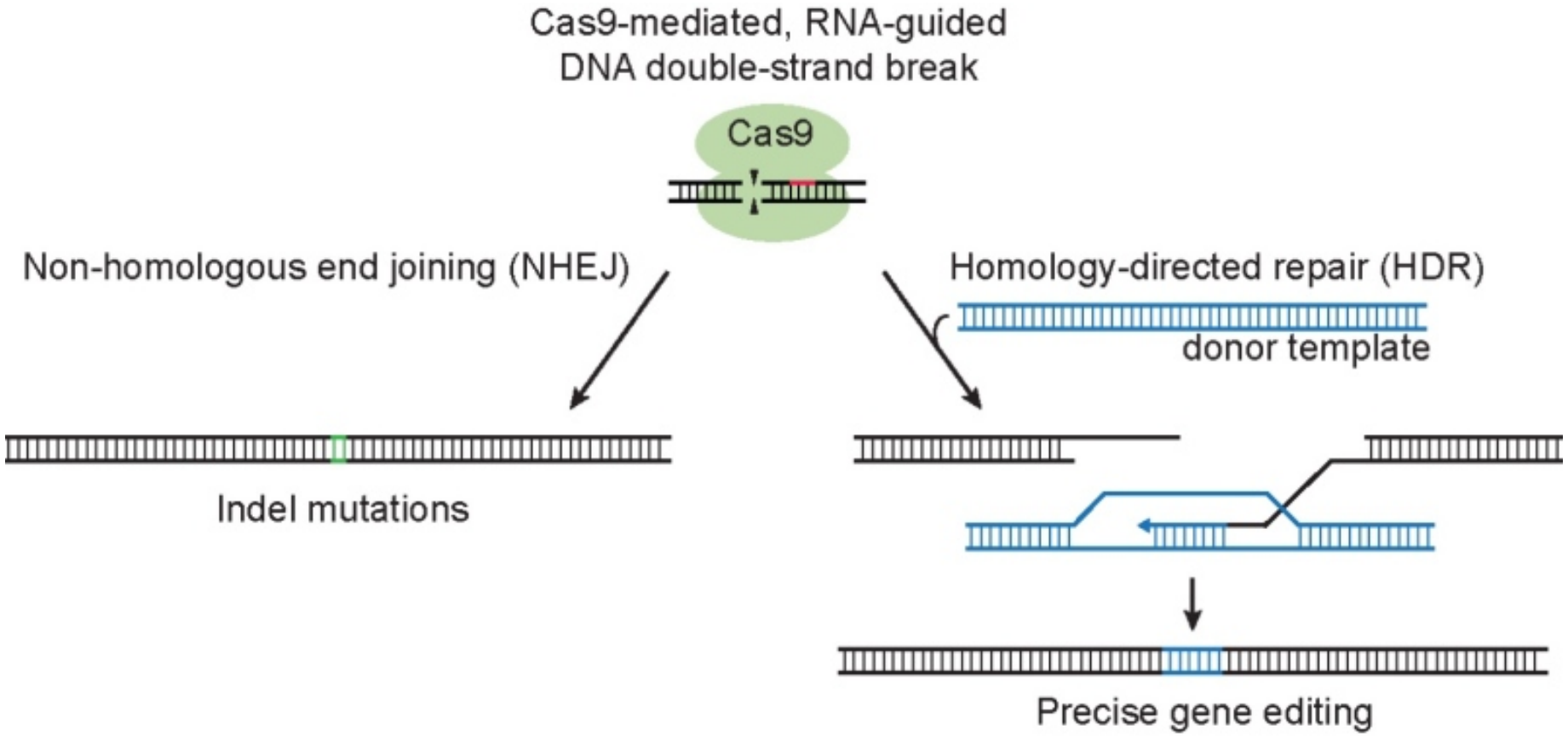
What happens if a dsDNA break occur?



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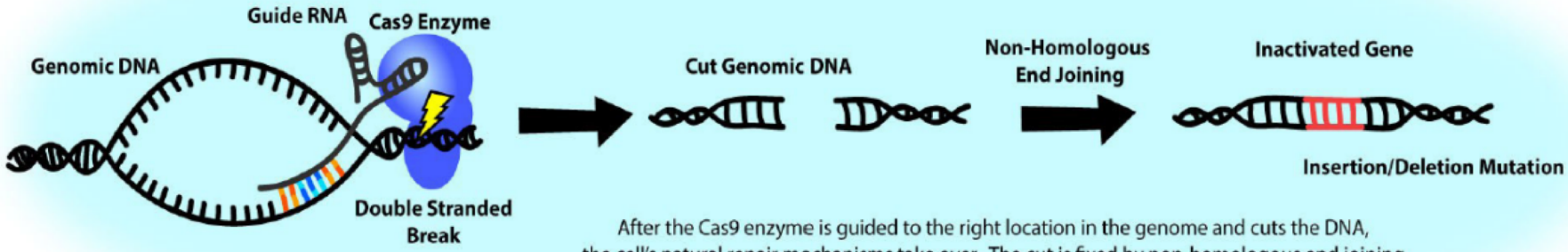
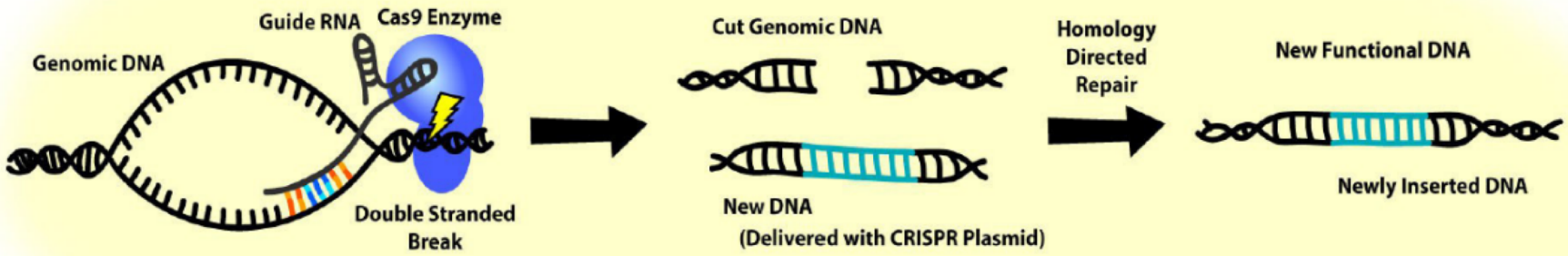
Gene Silencing with CRISPR



After the Cas9 enzyme is guided to the right location in the genome and cuts the DNA, the cell's natural repair mechanisms take over. The cut is fixed by non-homologous end joining. This process is error-prone and does not perfectly replace the cut DNA, often resulting in an insertion or deletion mutation which silences the gene.

Gene Insertion with CRISPR



To insert a gene, the new gene is added into the original CRISPR plasmid. It is designed to line up perfectly with the cut DNA strands, so the cell uses a different technique, homology directed repair, to incorporate a new stretch of DNA into the genome.

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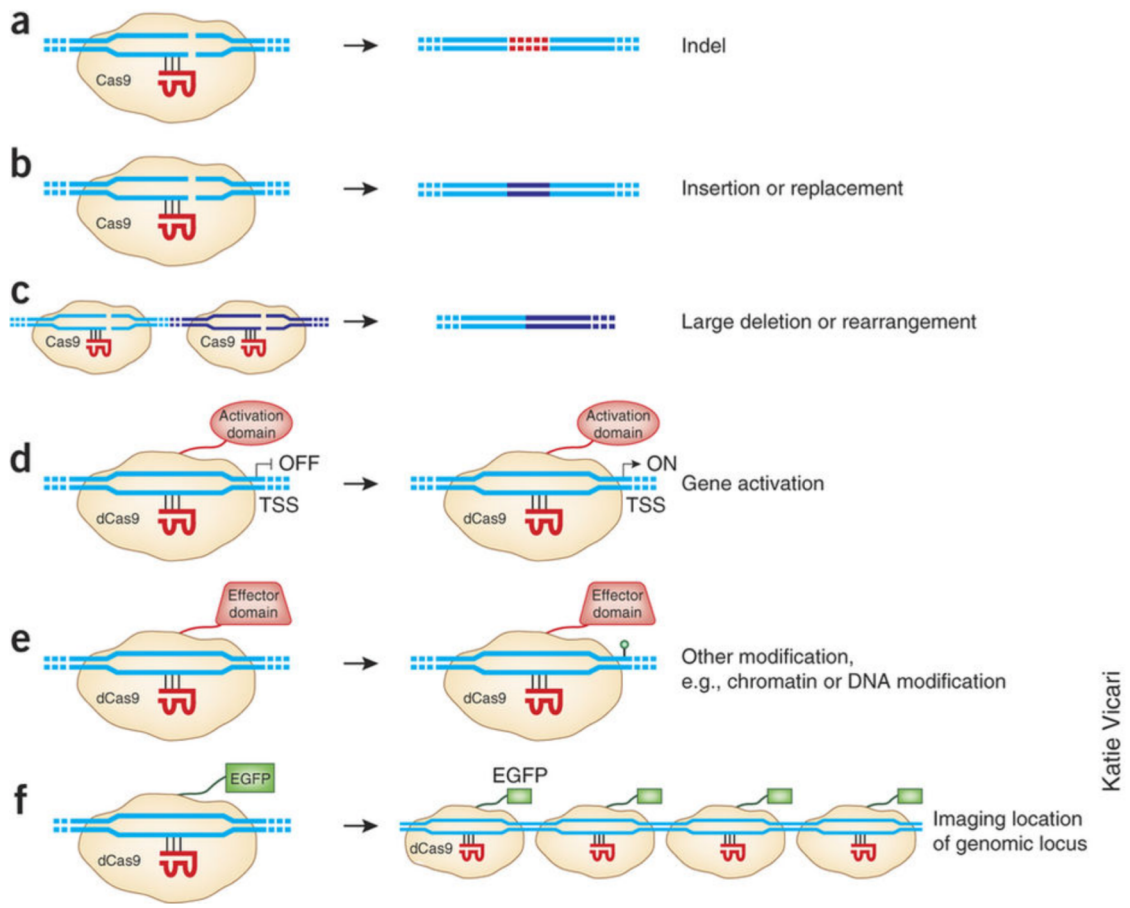
Versatile nature of CRISPR technology



(**a**,**b**) gRNA-directed Cas9 nuclease can induce indel mutations (**a**) or specific sequence replacement or insertion (**b**). (**c**) Pairs of gRNA-directed Cas9 nucleases can stimulate large deletions or genomic rearrangements (e.g., inversions or translocations). (**d**–**f**) gRNA-directed dCas9 can be fused to activation domains (**d**) to mediate upregulation of specific endogenous genes, heterologous effector domains (**e**) to alter histone modifications or DNA methylation, or fluorescent proteins (**f**) to enable imaging of specific genomic loci. TSS, transcription start site.

Jeffry et al., 2014
Nature Biotechnology

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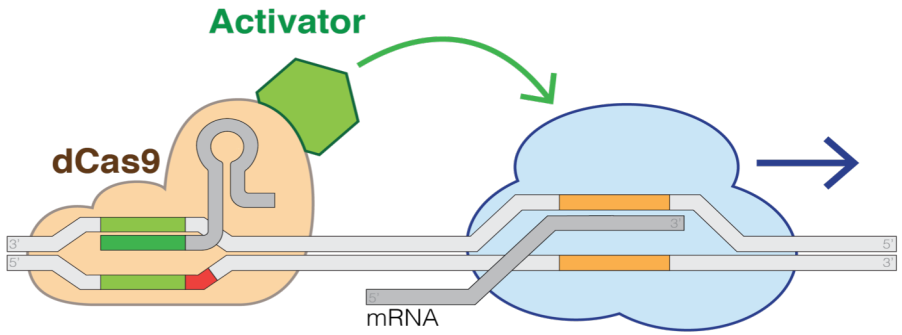
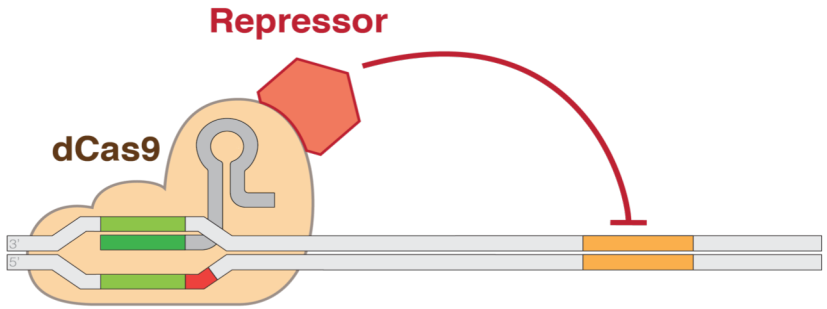
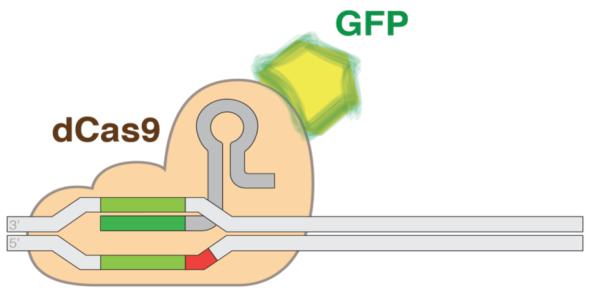
Cas9 modifications and their applications



Gene activation



Gene repression



Visualisation

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CRISPR-Cas: why it is so revolutionary?

- ✓ **Simple**: it only requires two components **gRNA and protein**
- ✓ **Precise**: can target any sequence in the genome –**base pairing**-
- ✓ **Universal**: it works in bacteria, plants, animals
- ✓ **Multitasking:** ability to target different sites at the same time -use multiple gRNAs-
- ✓ **Broad applicability** to both *in vivo* and *ex vivo* systems

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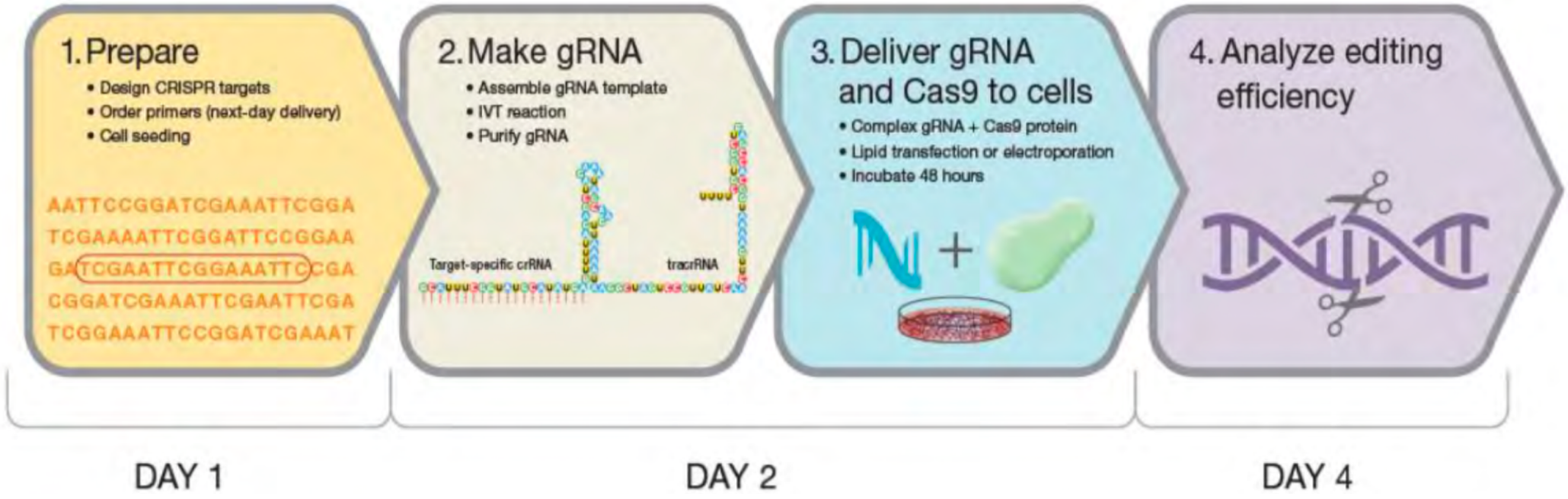
CRISPR reagents timeline: super fast !!!!!



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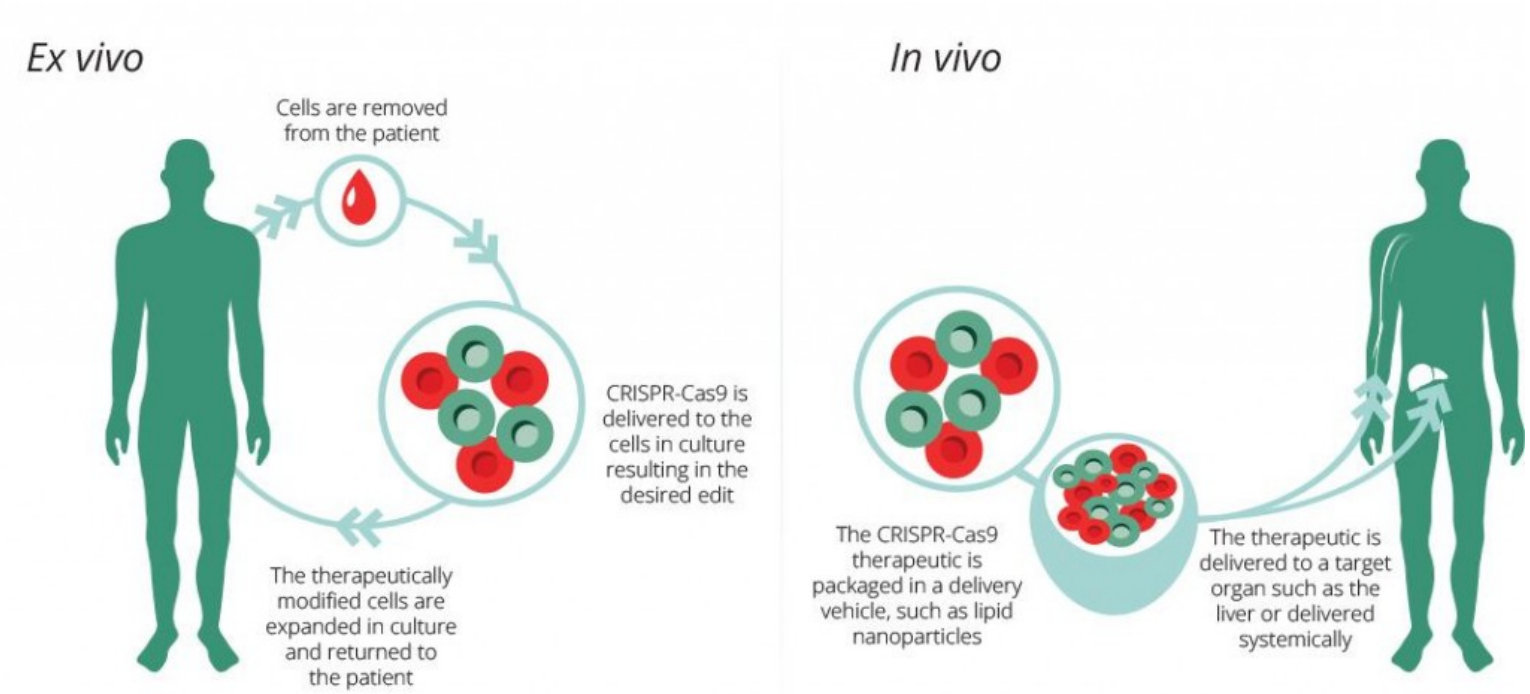
Therapeutic applications in humans



- Heritable diseases (*Cystic fibrosis, Duchenne muscular distrophy)*
- Cancer
- Immunological diseases

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CRISPR applications in Agriculture

- ✓ Potential tool for developing virus resistant crop varieties
- ✓ CRISPR can be used to eradicate unwanted species like herbicide resistant weeds, insect pest
- ✓ Developing biotic and abiotic resistant traits in plan

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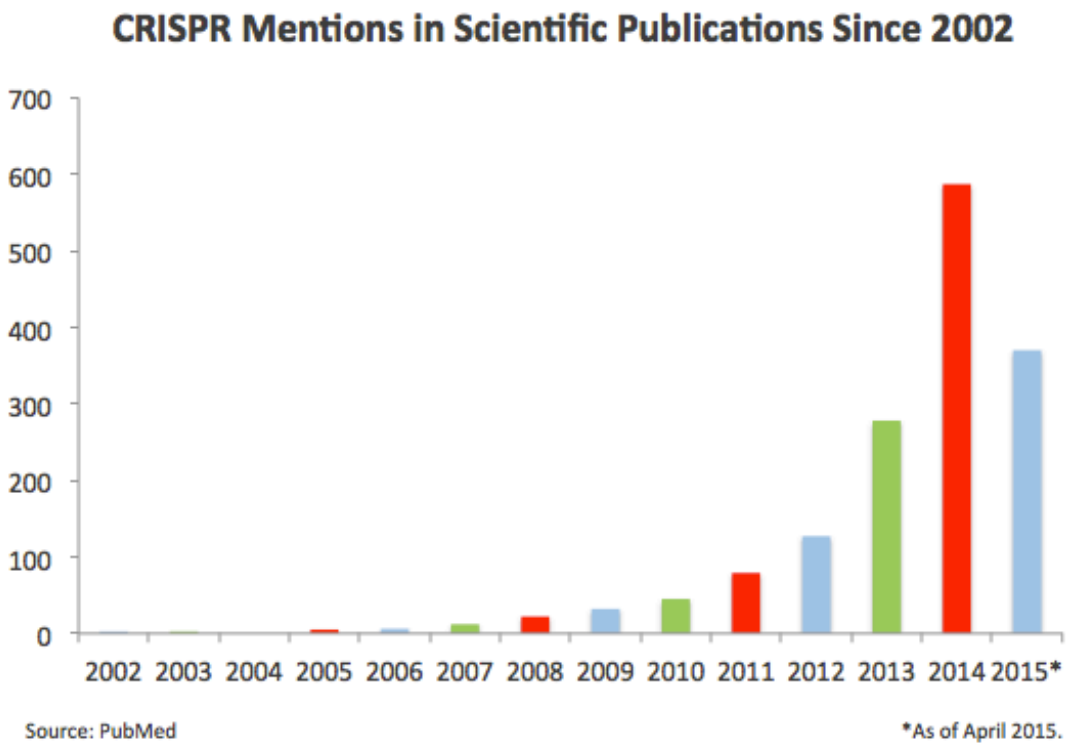
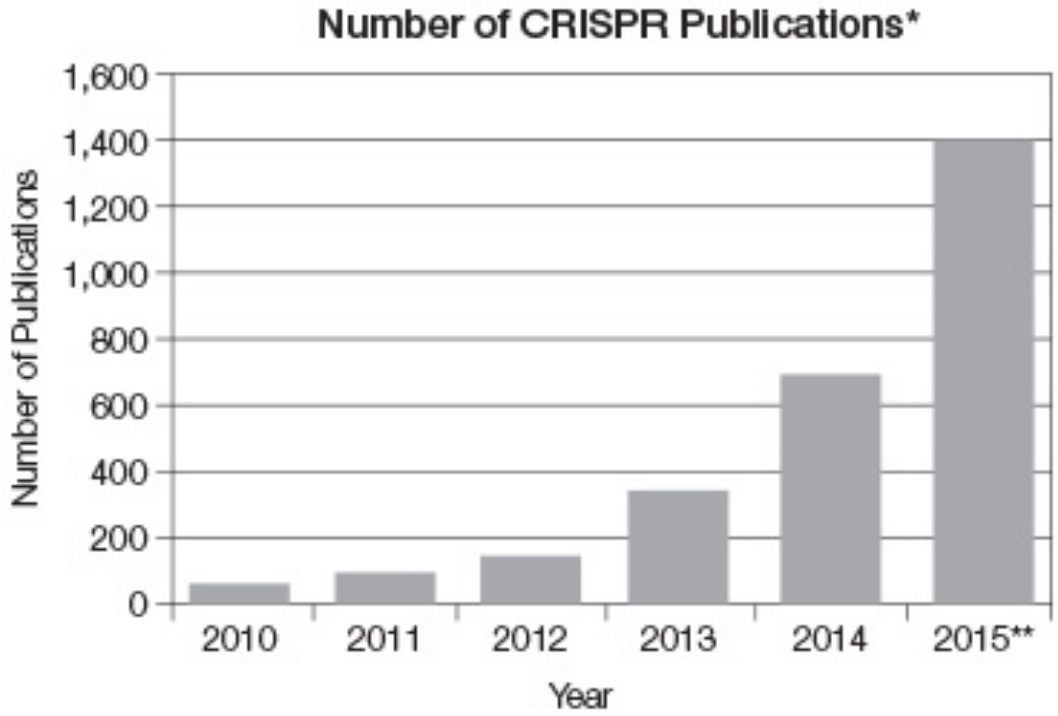
CRISPR impact on scientific publications





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CRISPR… the bad: OFF-TARGETS effects

Off-target predictor:

Chrom	Position	Strand	Sequence	Off-score	Gene	Region
chr07	21812180	-	AGAAGTGAGTTGGGAAACTA AGG	0.385		intergenic
chr02	303653	-	GGAACAGAGTGGGGAAACAA CGG	0.314		intergenic
chr04	24243692	-	AGAAGAGAGTCTAGAAACAA CGG	0.237	OS04G0485000	three_prime_UTR
chr06	10813090	+	AGGAGAGACTTGGGAAAAAA TGG	0.177	OS06G0294100	three_prime_UTR
chr01	10114801	-	ACTGGAGTGTTGGGAAACAA TGG	0.131	OS01G0283000	CDS
chr03	21759865	-	ATGGGAGAGTTGGCAAACAA TGG	0.104	OS03G0588800	intron
chr03	17285560	-	GGAAGTGAGTTGGGAAAGAA GGG	0.095		intergenic
chr11	20396137	-	GAAGGAGAGTTGGGAACCAA TGG	0.093		intergenic
chr07	17544625	-	AGAAGAGACTTGGCGAAAAA TGG	0.081		intergenic
chr07	16883830	-	AGAAGGGAGTTGGGAACCAT GGG	0.076	OS07G0471050	three_prime_UTR
chr07	19733721	-	AGAAGTGAGTTGGGAAAGAG GGG	0.073		intergenic
chr11	20185223	-	AGATGTGAGTTGGGAAAGAA GGG	0.061	OS11G0547000	five_prime_UTR

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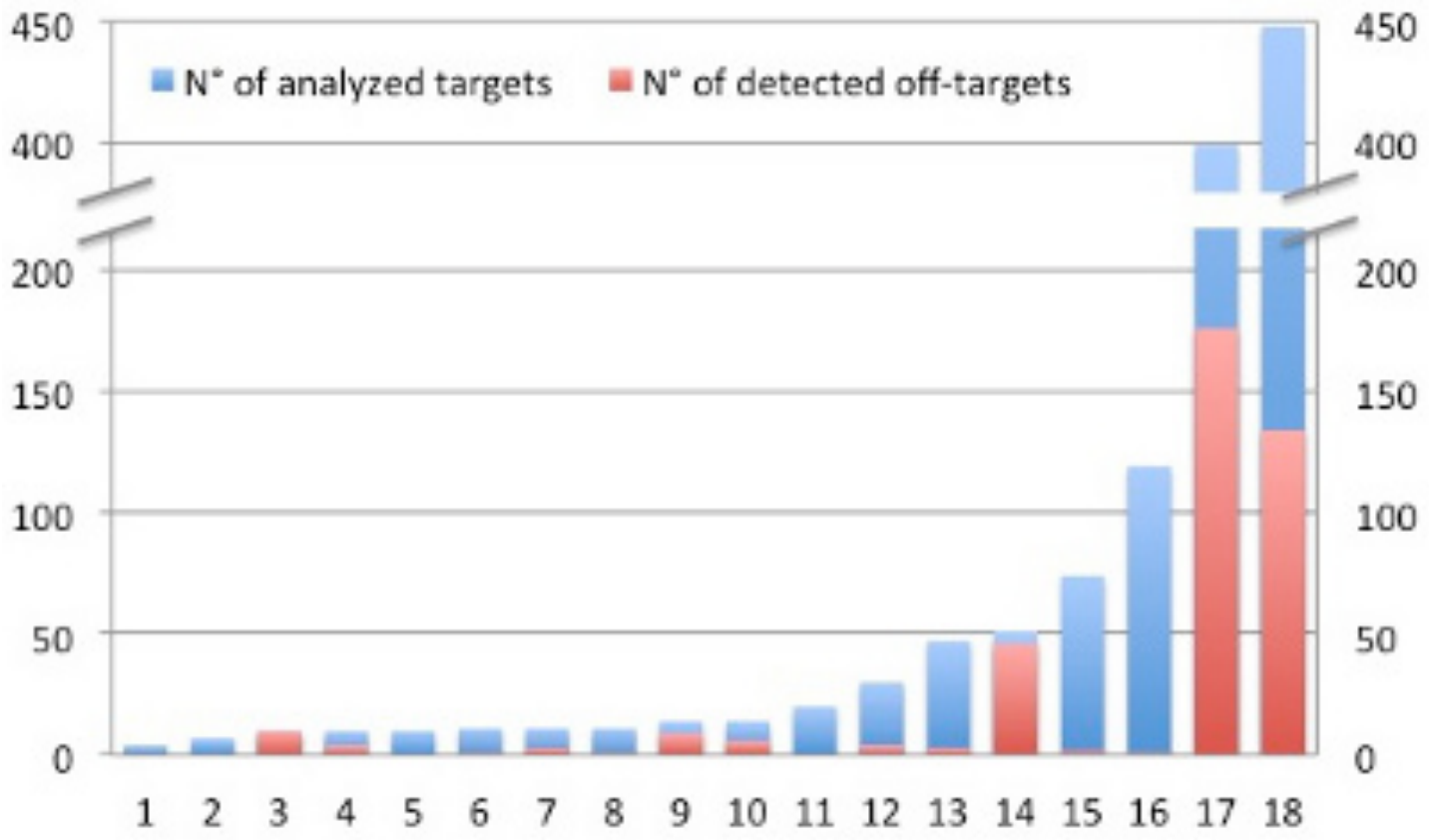
Multiple sites targeting: on target/off-targets effects



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Nature, March 2016

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