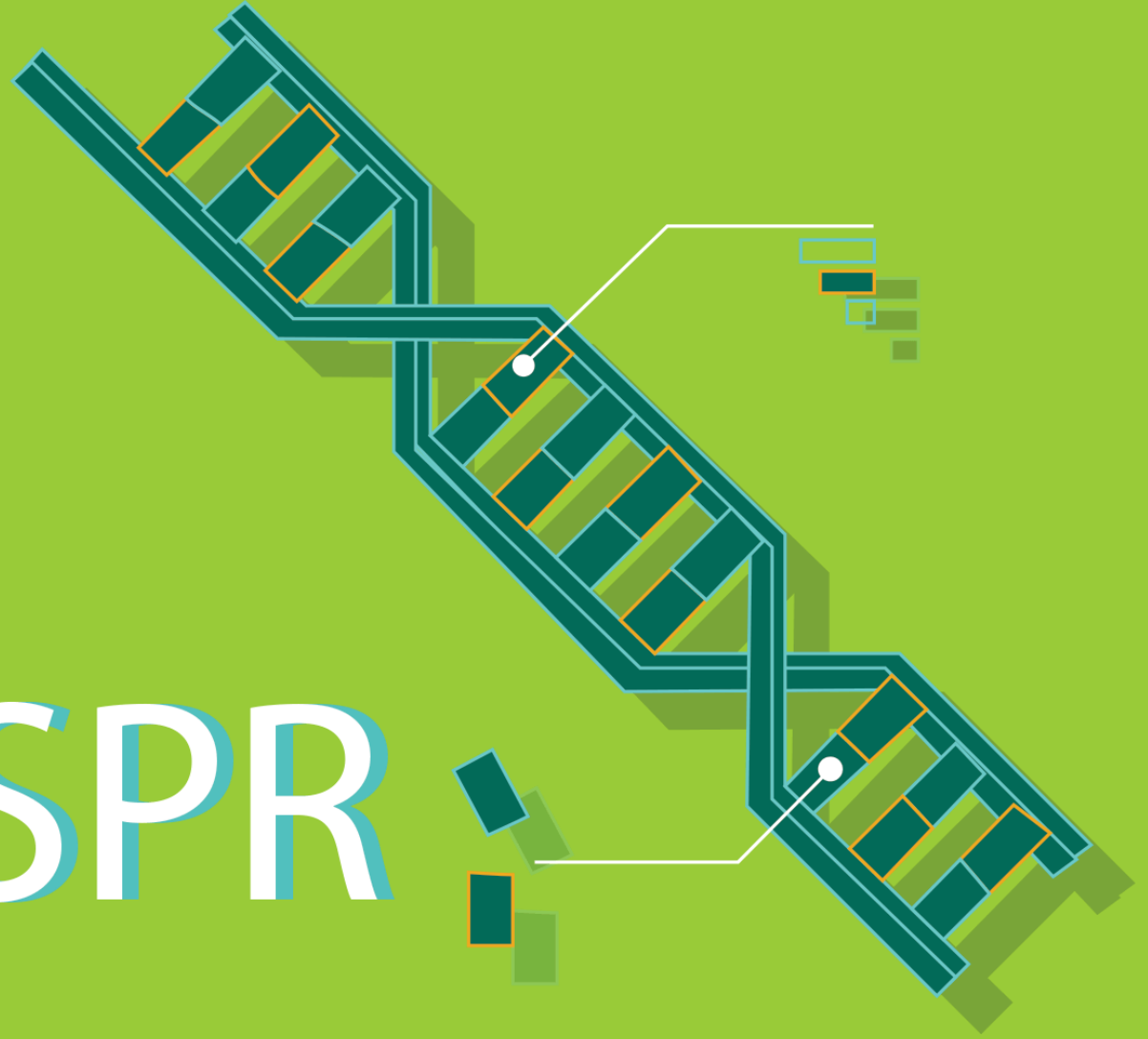
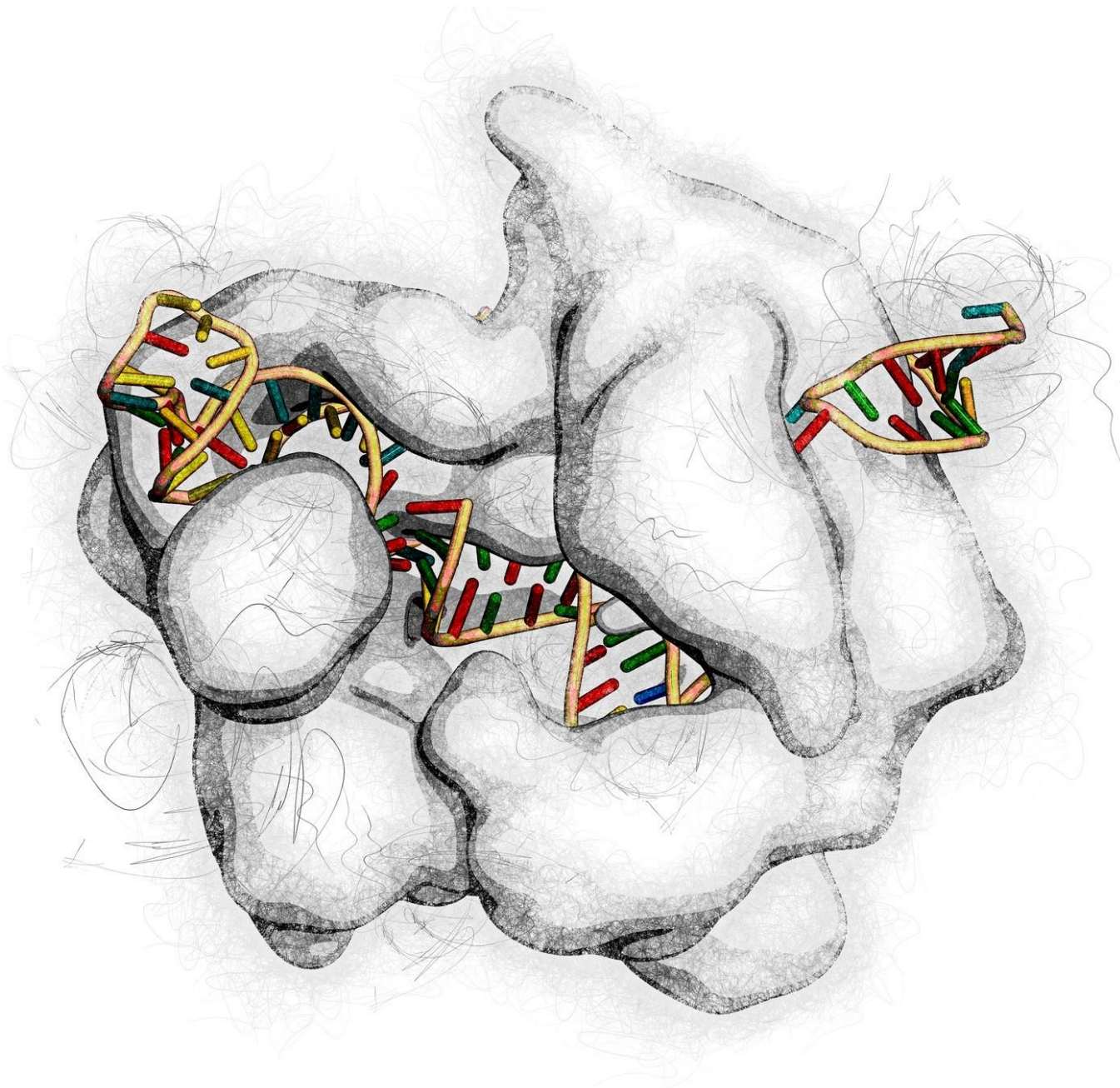


CRISPR





Index

- What is the Genome Editing?
 - Previous techniques
- What is CRISPR-CAS9?
 - How does it work?
 - Advantages
- History
- Classification
- Application
- Study Cases
- Future developments
- Ethical problem
- Bibliography and Sitography

Genome Editing

What (DEF)

Genome editing is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism.

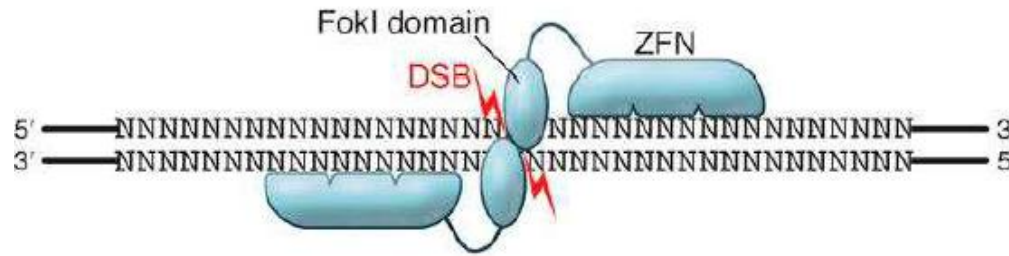
Unlike early genetic engineering techniques that randomly inserts genetic material into a host genome:

- Target the insertions to site specific locations → NUCLEASES: DSBs
- Repair pathways {
 - NHEJ: non-homologous end joining → indels (knock out)
 - HDR: homology directed repair → homology recombination donor (knock in)

How

1. { HR: homology recombinations
Mutagenesis site-directed } → No engineered nucleases
2. Meganuclease
3. ZFNs: Zinc finger nucleases
4. TALENs: Transcriptor activator-like effector nucleases
5. CRISPR

ZFN

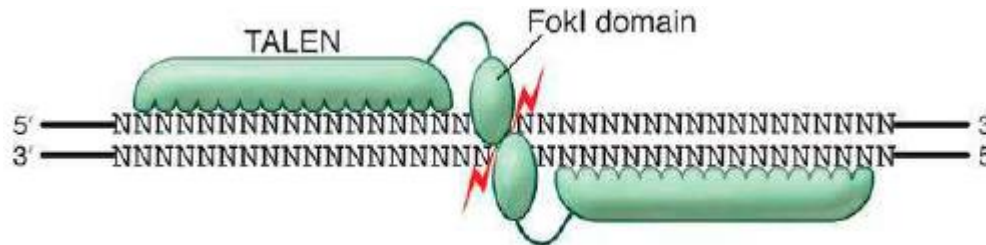


2 domains:

- Endonucleases (FokI enzyme)
- DNA binding (zinc finger motif)

- Time consuming
- Many difficulties: specificity of a module can change according to the modules fused to it

TALEN



2 domains:

- Endonucleases (FokI enzyme)
- DNA binding (TALE)

- Every single block recognize a single nucleotide
- Quick method
- Easier: no interference with the neighboring modules

CRISPR/Cas: a spreading revolution

Clustered Regularly Interspaced Short Palindromic Repeats

- CRISPR systems: 3 different types (same working principle although different components)

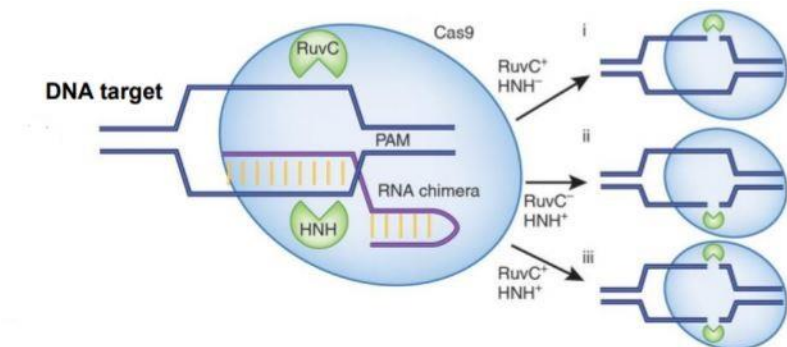
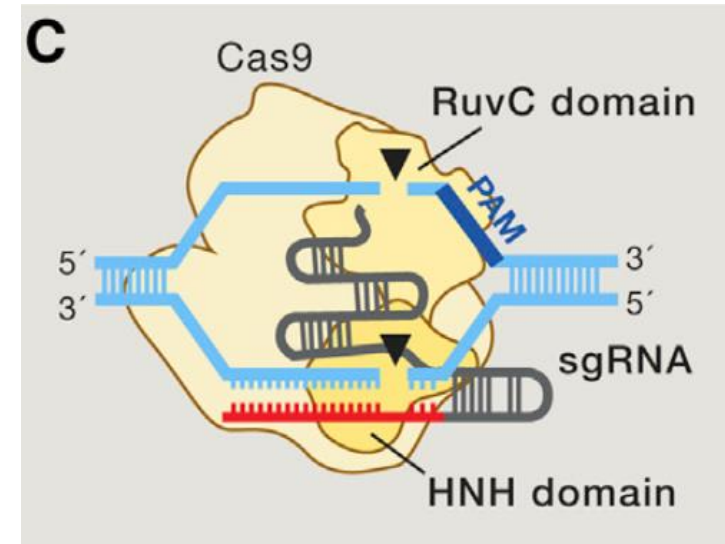
➤ **CRISPR Cas9 type II**, most widely utilized in genome editing studies/applications

- **Components:**

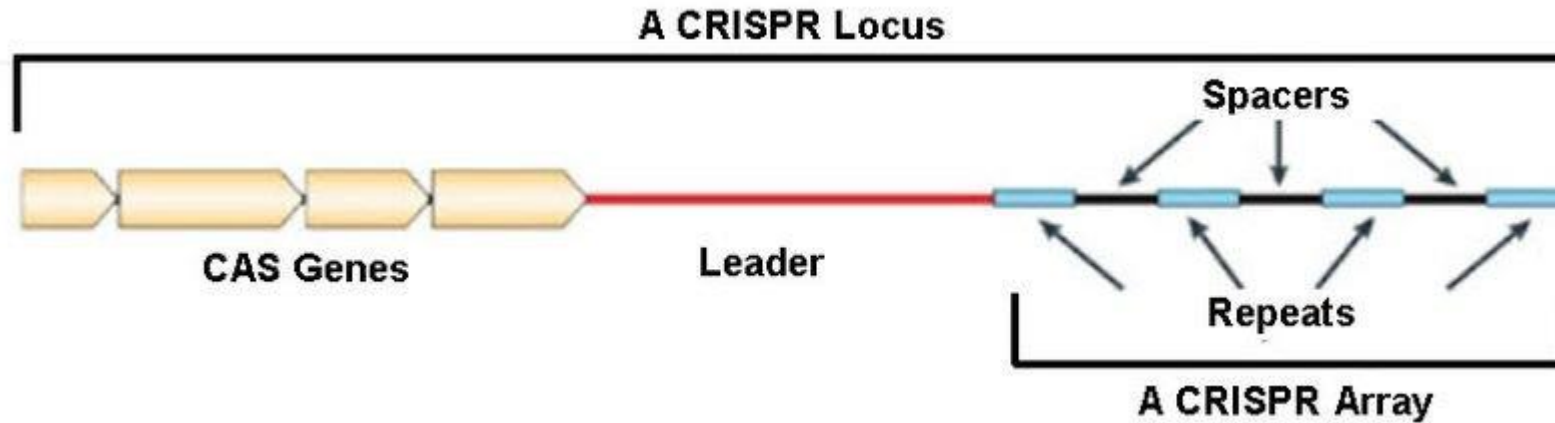
- Cas9 endonuclease ➡ RuvC & HNH domains
- gRNA ➡ Target sequence detection via PAM

2-6 base pairs DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease.

Cas9 orientation ←



Into bacterial nucleus: CRISPR locus



Spacers DNA: region of 30 pair of viruses DNA bases incorporated between genes (palindromic **repeats**)

- Like a ROM memory: preserves all the viruses DNA that previously attacked the bacterium
- During a new viral attack, RNA copies of bacterial DNA complex with Cas9 protein

tracrRNA (from leader sequence)



Scaffold sequence that binds to Cas9 protein

crRNA (from CRISPR ARRAY)



20 nts sequence, designing component

hybridization



gRNA

➤ **Working principle** → adaptive immunity in bacteria [Credits to: [McGovern Institute for Brain Research at MIT](#)]



Pro & Cons

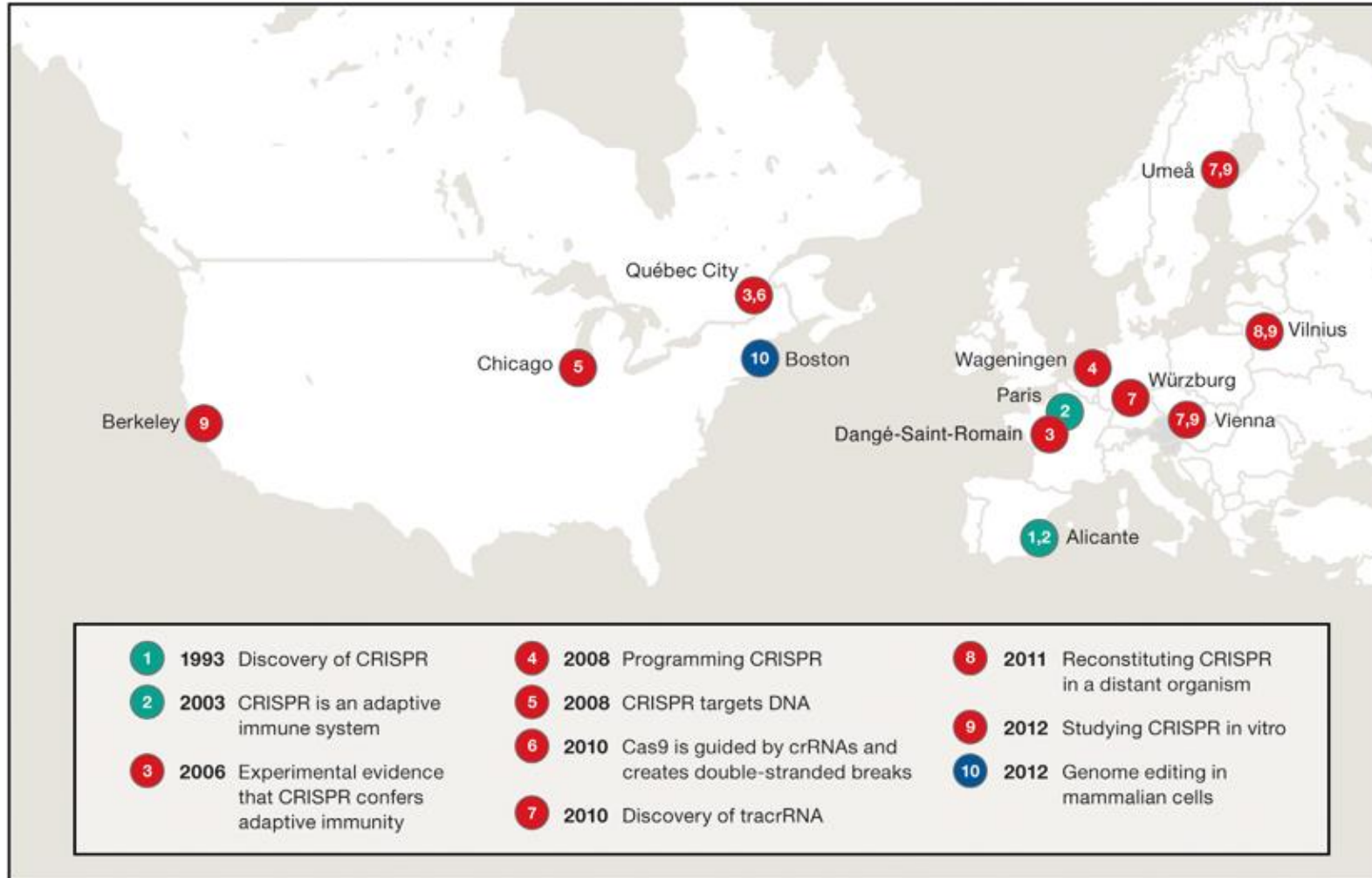
- ✓ Universality
- ✓ Versatile
- ✓ Unexpensive (10^2 vs 10^3 €)
- ✓ Simple & quick (days vs weeks)
- ✓ High precision targeting (still to be improved)
- ✓ Multiplexing
- ✓ Comprehension of the different functions that gene of interest plays (activating or silencing gene expression)

- ✗ Off-target cleavage
- ✗ High [Cas9] → higher freq. off-target cleavage
- ✗ Further informations needed (solving black-boxes & viruses long-term effects)

The History of CRISPR-Cas9

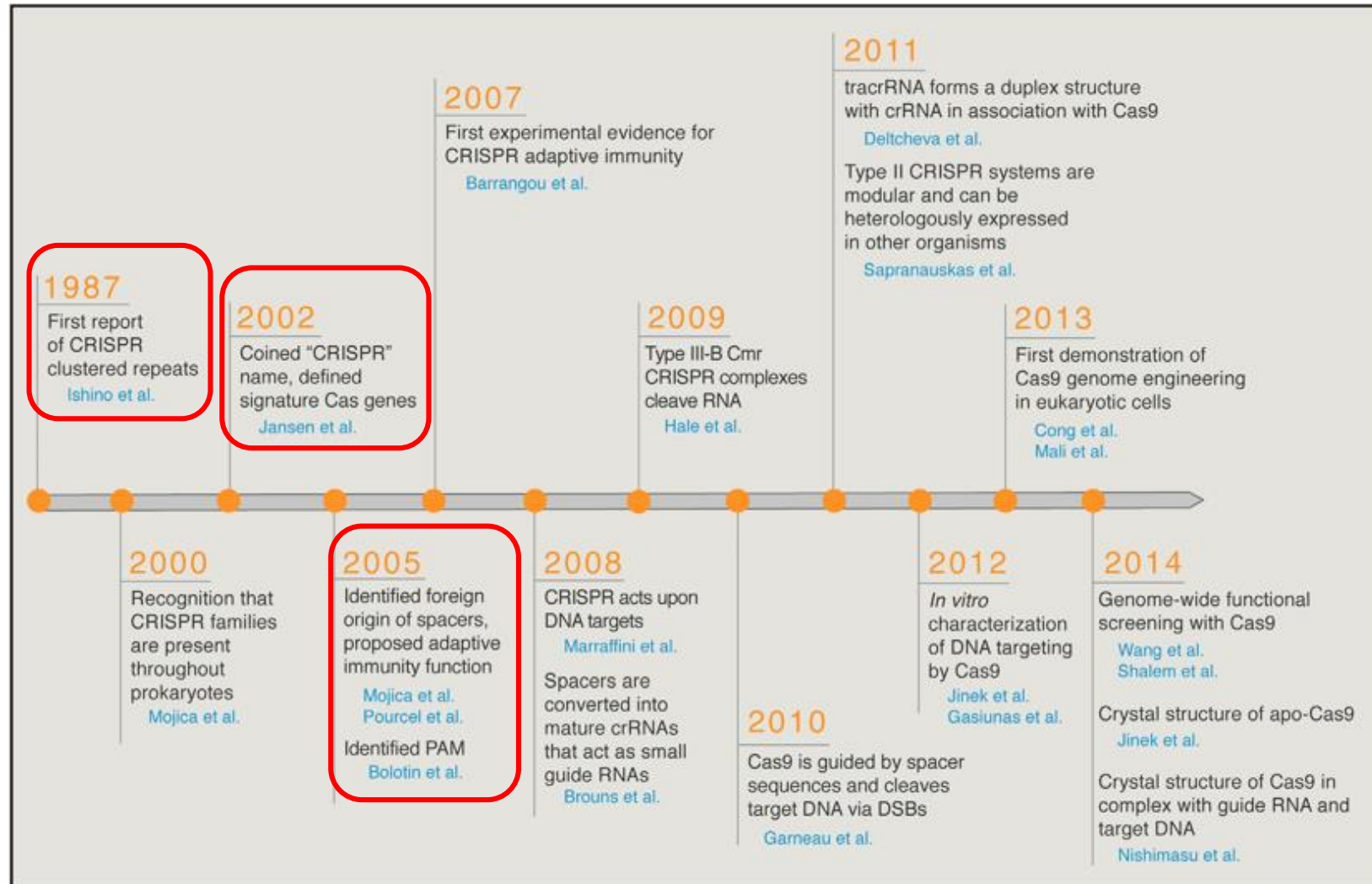
A Journey through Modern Scientific Research

The History of CRISPR-Cas9: A Journey through Modern Scientific Research



- Originally discovered in bacteria and later used as an efficient tool for genome editing
- Over 20 years of researches across many countries and different research fields
- Extensive use of bio-informatics and complex molecular biology techniques

The History of CRISPR-Cas9: An Adaptive Immune System in Bacteria



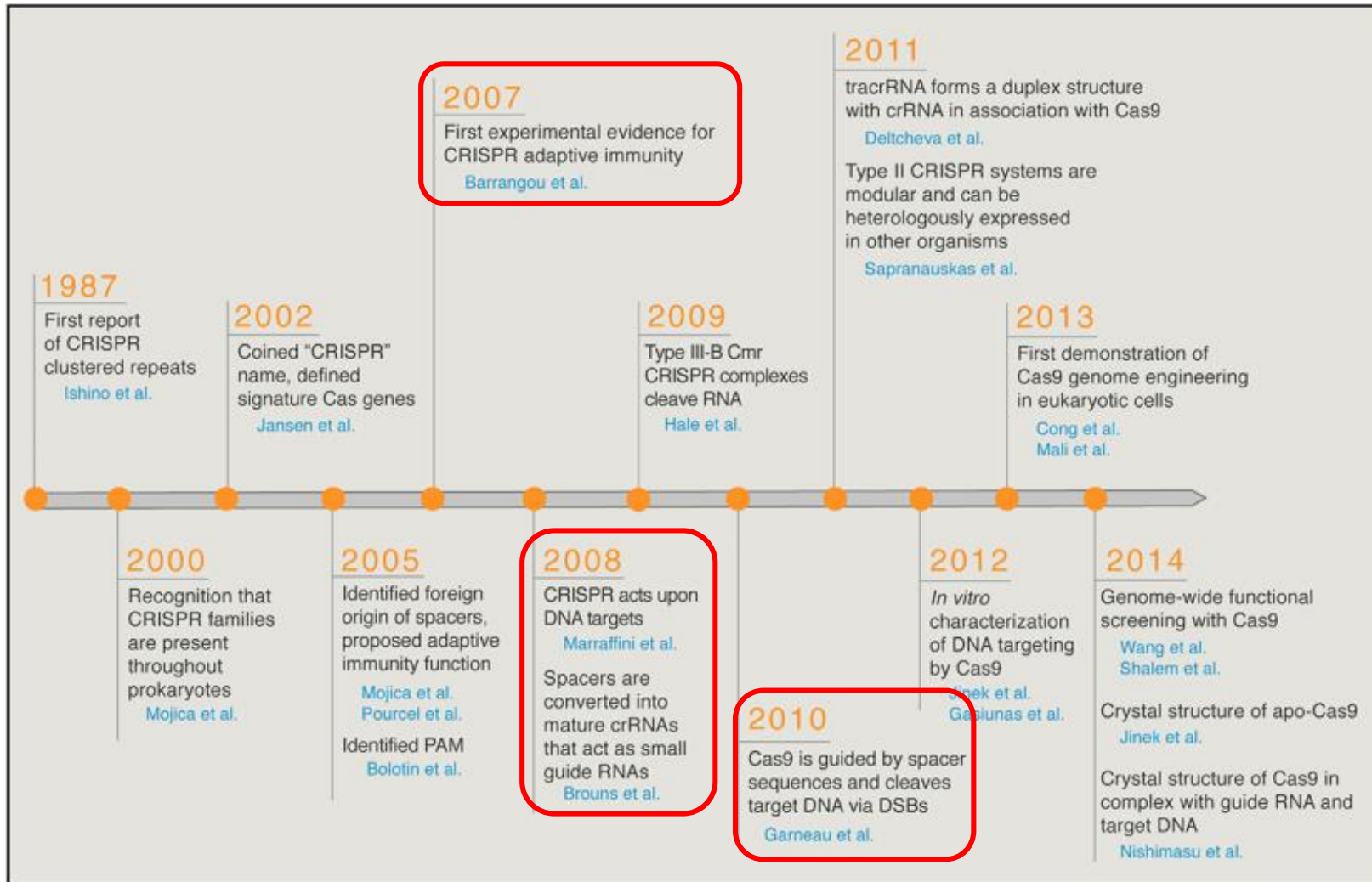
Three main research groups:

- ✓ Mojica et al. (Alicante University)
- ✓ Vergnaud, Pourcel et al. (French Ministry of Defense)
- ✓ Bolotin et al. (French National Institute for Agricultural Research)



"CRISPR locus serves in a defense mechanism as it may represent a memory of past genetic aggressions"

The History of CRISPR-Cas9: How does it work?

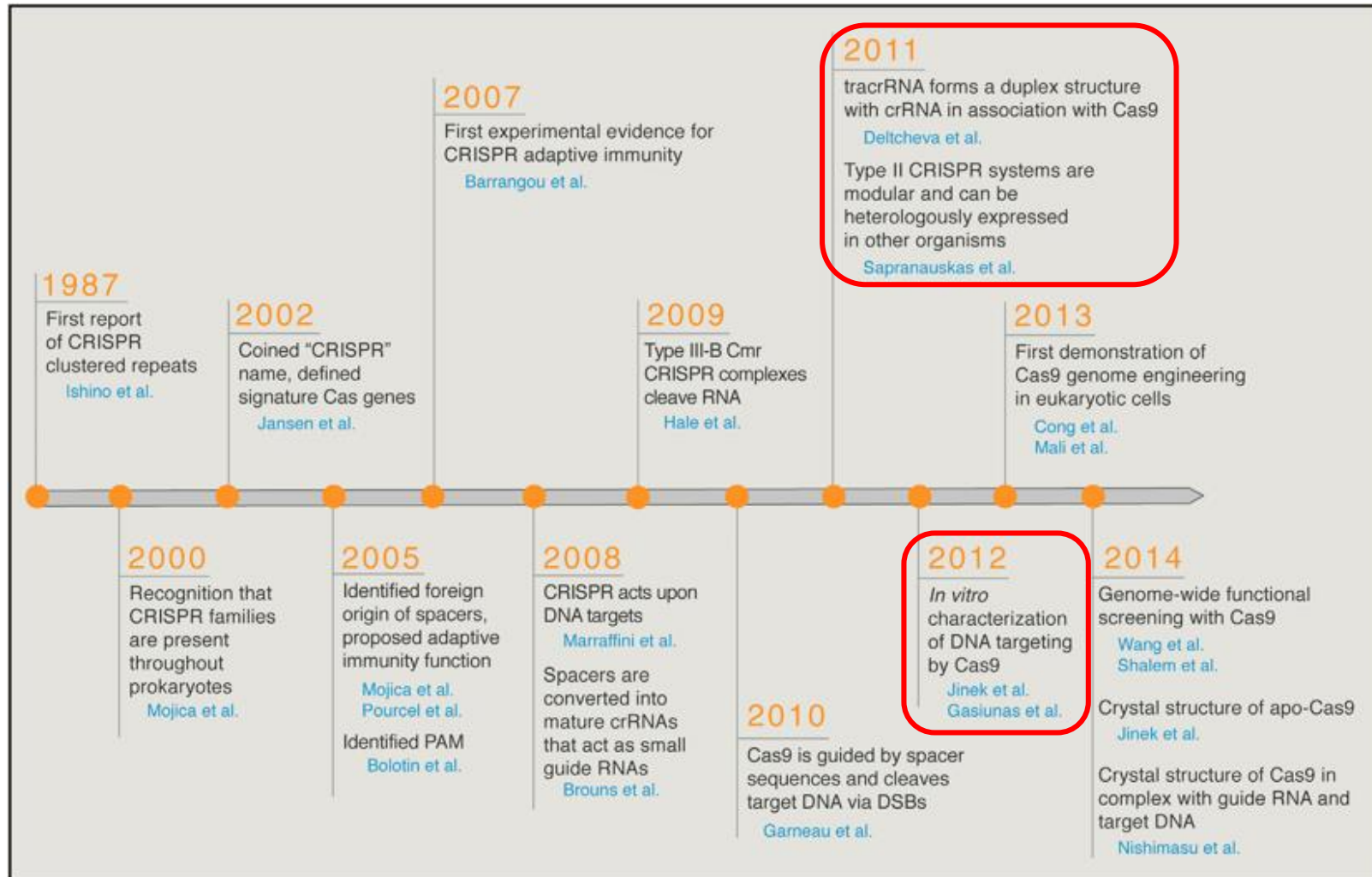


- ✓ Hovarth et al.
(Danisco in France)
- ✓ Van der Oost et al.
(Wageningen University)
- ✓ Marraffini and Sontheimer
(Chicago University)
- ✓ Moineau et al.
(Danisco in France)



“The ability to direct the specific addressable destruction of DNA that contains any given target sequence could have considerable functional utility”

The History of CRISPR-Cas9: The Last Missing Piece of the Puzzle

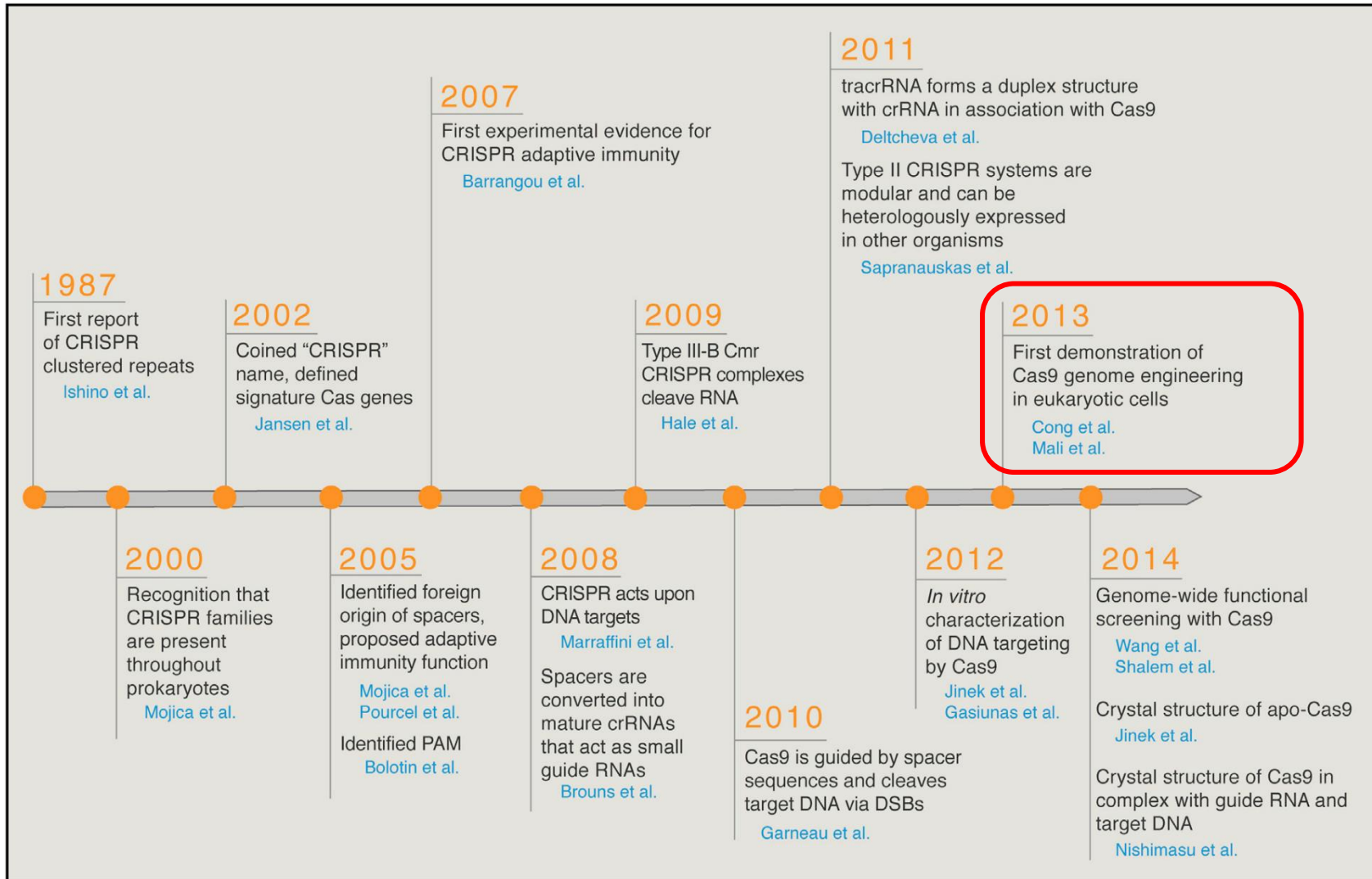


- ✓ Charpentier et al. (Würzburg University)
- ✓ Syksnys et al. (Vilnius University)



“The necessary and sufficient components of the CRISPR-Cas9 system were now known. It was now time to turn to precise biochemical experiments to try to confirm and extend the results in a test tube”

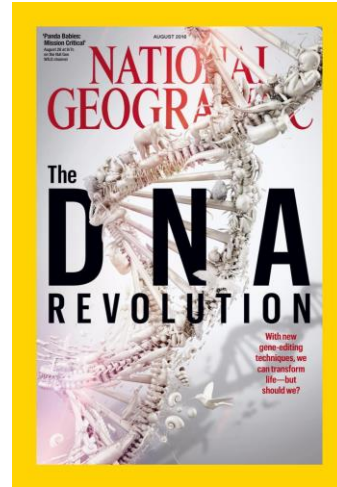
The History of CRISPR-Cas9: In Vitro experiments and Genome Editing



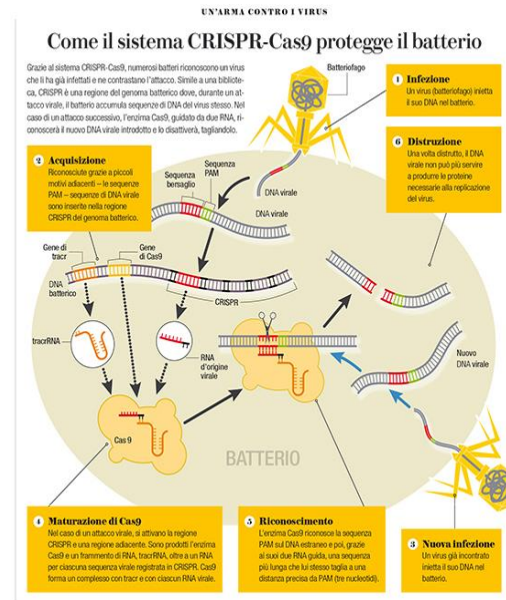
- ✓ Charpentier et al. (Würzburg University)
- ✓ Syksnys et al. (Vilnius University)
- ✓ Zhang et al. (MIT)
- ✓ Church et al. (Harvard University)

and many more...

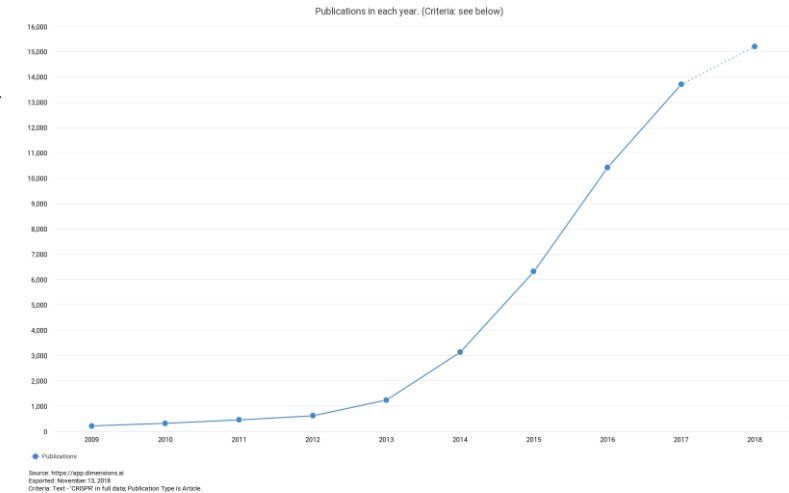
The History of CRISPR-Cas9: CRISPR goes Viral



RE LE INCHIESTE



Fonte: Le Scienze



Interesse nel tempo

Google Trends

● CRISPR



Applications of CRISPR-based technologies

Applications of CRISPR-based technologies

CRISPR it's a tool of surgical precision mutagenesis and, at the same time, easily transferable at large scale.

There are various applications of CRISPR technologies, for example in agricultural biotechnologies, industrial technologies and biomedical technologies.

The agricultural biotechnologies have been the trailblazers: corn, soya and wheat modified with CRISPR have already been produced by different companies and could soon be on the market. Compared to the more traditional GMOs, these seeds do not carry foreign genes in their DNA and thus escape many of the restrictions that make the transgenic products a bottleneck.

In BIOMEDICAL ENGINEERING:

CRISPR-based technologies have empowered researchers with an unprecedented toolbox that enables breakthrough discoveries and innovative methodologies.

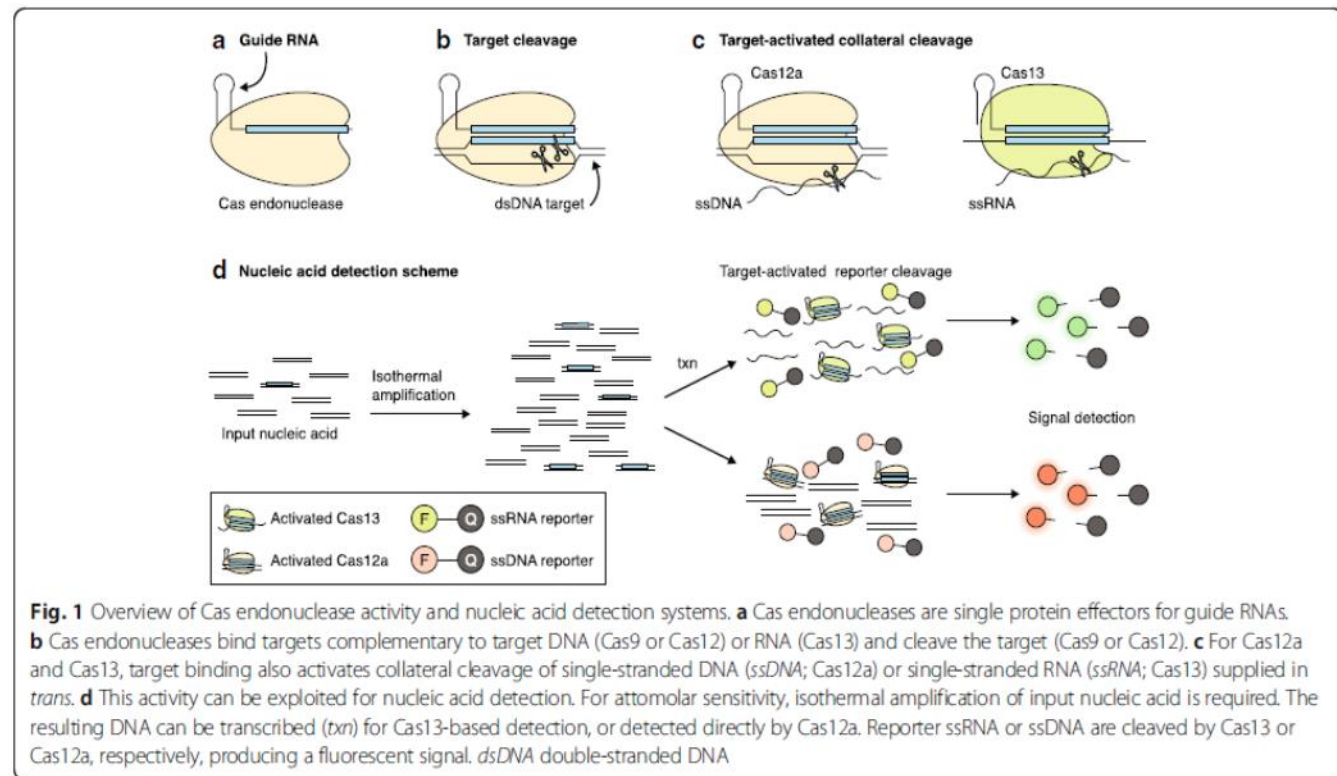
Specially CRISPR shows incredible promise as a platform for studying gene function in vivo.

These methodologies could expand their therapeutic applications, such as the PATHOGEN DETECTION, DISEASE MODELING, and most importantly DISEASE THERAPY.

PATHOGEN DETECTION

The discovery of Cas proteins with alternative activities has now enabled sensitive and robust tools for detecting nucleic acids.

CRISPR–Cas technologies display their potential for providing low-cost and practical diagnostic tools for pathogen and disease detection.



DISEASE MODELING IN VIVO

MOUSE MODELS

The mouse as a model organism has been at the forefront of genetic engineering since its inception and has, perhaps not surprisingly, led much of the research on nuclease-directed genome editing. Accordingly, CRISPR has been quickly embraced by the mouse modelling community, bringing genomic manipulation to a new level of flexibility.

The main types of mouse models are: GERMLINE, TRANSPLANTATION, EXOGENOUS DELIVERY, INDUCIBLE MODELS, MODELING THE COMPLEX GENOMA.

NON-MAMMALIAN MODELS

CRISPR technology is not only the domain of the mouse. Even genetically-pliable models such as zebrafish, *Caenorhabditis elegans*, and *Drosophila* have benefited from CRISPR-driven genome modification.

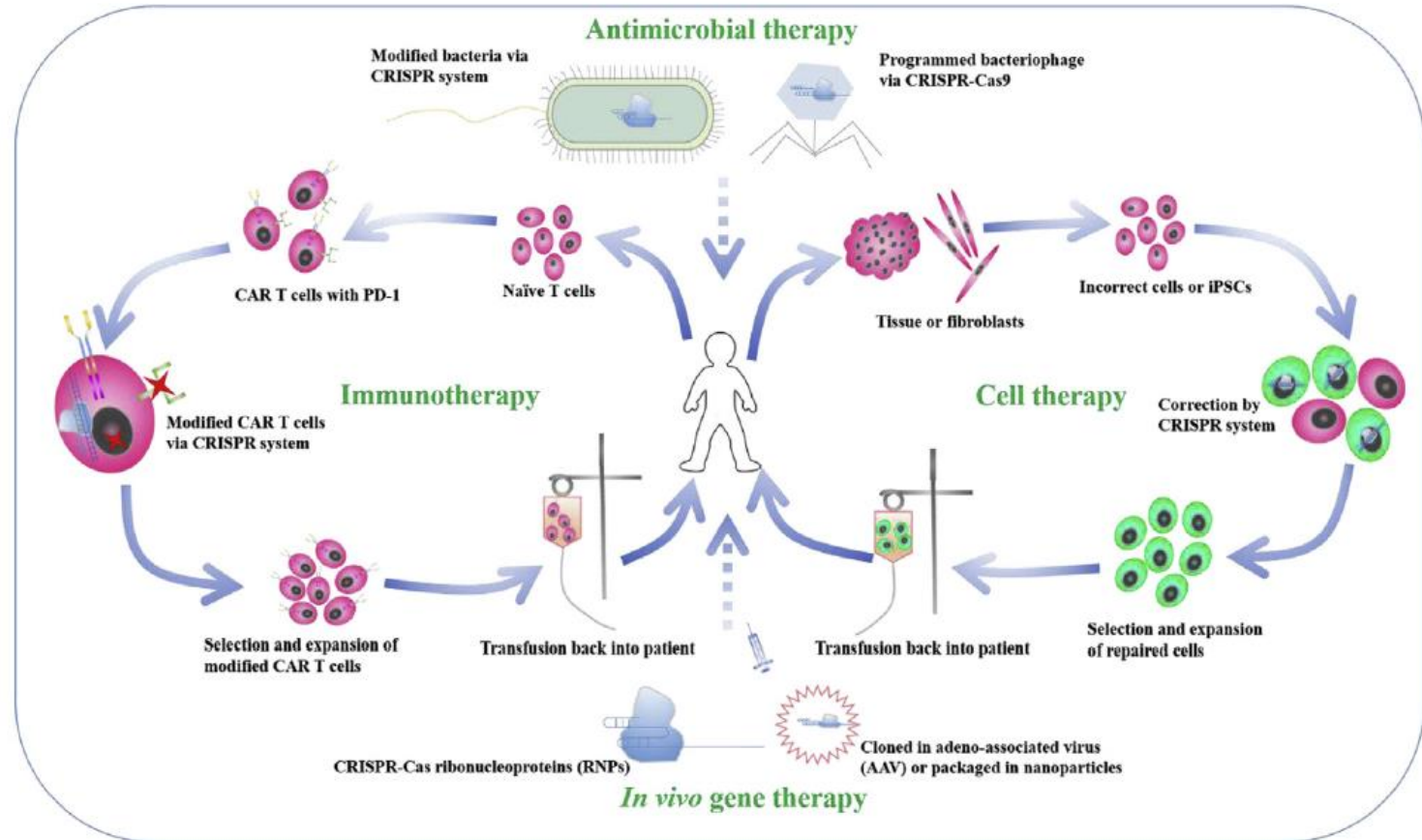
LARGER MAMMALS

Where genome-editing tools, in particular CRISPR technology, show promise to be truly revolutionary is in larger vertebrate model systems. These include mice, where CRISPR is changing the landscape of genetic analysis (see above), but also rats, pigs, and non-human primates, where traditional genetic tools developed in mouse have been difficult to adapt.

DISEASE THERAPY

An overview:

The CRISPR systems can be used for in vivo gene therapy through the Cas ribonucleoproteins (RNPs) or delivered by AAV. Naïve T cells come from the patients and CAR T cells are activated by antibody-coated beads. The reprogrammed CAR-T cells generated by knocking out the PD-1 or other receptors, were transfused back to the patients. The incorrect cells are isolated from the patients and iPSCs are generated by reprogramming the fibroblasts, and corrected with the help of CRISPR-based technologies. The repaired cells were used for therapy.



IN VIVO GENE THERAPY

Gene therapy is defined as the delivery of nucleic acid into the patients for disease treatment.

Traditional gene therapy strategies include interfering with target gene expression, replacing the culprit genes, correcting genetic mutations and so on.

With the development of CRISPR technologies, the recent advances in gene therapy intrigued our great interests.

EXAMPLES:

- In 2014, the Fah mutation which causes human hereditary disease tyrosinemia was corrected in adult mice via CRISPR-Cas9.
- In the same year, the CRISPR-Cas9 was successfully introduced into mice liver through adenovirus packaging and mutated the endogenous proprotein convertase subtilisin/kexin type 9 (PCSK9) gene with high efficiency. These mutations of Pcsk9 reduced the low-density lipoprotein cholesterol level and protected against cardiovascular disease.
- On 30th August 2018 a muscular dystrophy was fixed in dog by CRISPR-based therapy.

CELL THERAPY

The easily accessible and programmable features of CRISPR-Cas system also spur a revolution in cell therapy. CRISPR technology was first applied in cell therapy in 2013, correcting the cystic fibrosis transmembrane conductor receptor (CFTR) locus in the cultured intestinal stem cells of cystic fibrosis (CF) patients.

Other than treating CF patients, CRISPR-Cas system has also been applied in the engineered human induced pluripotent stem cells (iPSCs) to correct disease-causing mutations, such as the β -thalassemia#

ANTIMICROBIAL THERAPY

In 2014, a bacteriophage system containing CRISPR-Cas9 was reported to deal with the antibiotic resistance. The modified bacteriophage targeted the antibiotic resistance genes of *Staphylococcus aureus*, and then destroyed the genes and immunized avirulent *Staphylococci* to prevent the spread of plasmid-borne resistance genes. Other than killing bacteria by bacteriophage directly, they designed the phages harbouring the CRISPR-Cas system to invade in the bacteria by lysogenization, which targets the specific DNAs of the bacteria such as the antibiotic resistance gene. If the bacteria contain the resistance gene, the gene will be cleaved by the CRISPR-Cas system, and the antibiotic sensitivity of the bacteria would be higher. This allows programming of lytic phages to kill only specific antibiotic-resistant bacteria.

IMMUNOTHERAPY

Being a powerful tool of genome editing, CRISPR technology holds great promise as the means for immunotherapy, especially chimeric antigen receptor (CAR) T cell-based adoptive immunotherapy.

T cells have the potential to target and destroy cancer cells.

However, the programmed death-1 (PD-1) receptor on activated T cells can bind with the ligand PD-L1 expressed on dendritic cells (DCs) or some tumor cells, thus, decreasing the activity of T cells.

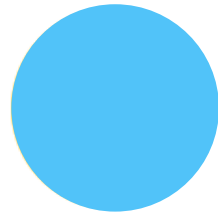
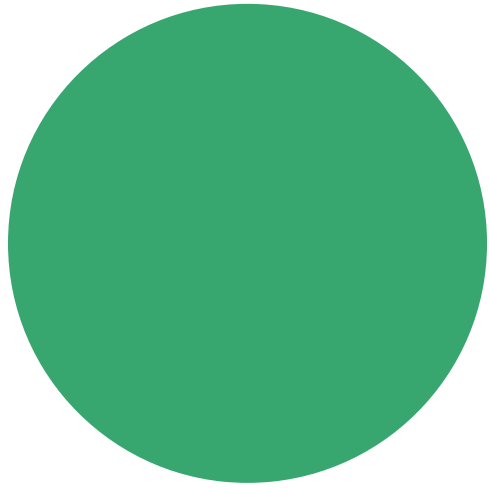
To solve this problem, CRISPR-Cas9 system has been employed to reprogram the primary human T cells, the endogenous T cell receptor (TCR) locus was replaced with a new TCR in order to recognize the cancer antigen easily via CRISPR-Cas9. The engineered CAR-T cells improved the anti-tumor efficacy by ablating the genes of PD-1 via CRISPR-Cas9 system. Due to the drawbacks of PD-1, CAR-T cells may recognize the alloantigens of the recipient, leading to graft-versus-host disease (GVHD), and may cause immunogenicity or alloreactivity because of HLA class I (HLA-I) on the surface of allogeneic T cells.

To eliminate these defects, multiplex genes were edited in CAR-T cells via CRISPR-Cas9, such as endogenous TCR locus (TRAC or TRBC) and β -2 microglobulin (B2M). The therapeutic efficacy of CAR-T cells with the lymphocyte activation gene-3 (LAG-3) deleted is higher than that of the untreated cells. In another case, the CAR was integrated into the TRC locus precisely under the control of endogenous regulatory elements, which can reduce tonic signaling, avert accelerated T-cell differentiation and exhaustion, and also increase the therapeutic potency of engineered T cells.

In clinic, the immunotherapy with the modified T cells by CRISPR-Cas9 is on the way.

Examples of disease treatment via CRISPR-based technologies.

Disease	Species	Possible pathogenesis	Therapeutic Method	Results
Hereditary tyrosinemia	mouse	Mutations of the fumarylacetoacetate hydrolase gene <i>Fah</i>	Delivery of the CRISPR-Cas9 system and the ssDNA donor into the mouse to correct the <i>Fah</i> mutants	Initial expression of the wild-type FAH protein in ~1/250 liver cells and rescued the body weight loss phenotype.
Cardiovascular disease	mouse	Mutations in the subtilisin/kexin type 9 gene <i>Pcsk9</i>	Delivery of adenovirus expressing Cas9/sgRNA targeting the <i>Pcsk9</i> gene in mouse liver	The mutagenesis correction rate of <i>Pcsk9</i> in the liver was > 50%
Duchenne muscular dystrophy (DMD)	mouse	Mutations in the dystrophin gene	Delivery of the SpCas9 or SaCas9/sgRNA targeting the exon 23 into the <i>mdx</i> mouse model	Excision of intervening DNA and restored the <i>Dmd</i> reading frame
DMD	mouse	Mutations in the dystrophin gene	Electroporation-mediated transfection of the Cas9/gRNA constructs targeting the exon 23 into the skeletal muscles of the <i>mdx</i> mice	Excision of the mutant exon 23 of <i>mdx</i> the <i>mdx</i> mice model
DMD	mouse	Mutations in the dystrophin gene	Injection of the LbCas12a mRNA, the sgRNA and the ssODN donor targeting the exon 23 into the <i>mdx</i> zygotes	The <i>DMD</i> mutations were corrected in the <i>mdx</i> mice model
DMD	canine	Mutations in the dystrophin gene	Delivery of the AAV vectors carrying SpCas9/sgRNA targeting the exon 51 into the cranial tibialis muscles	Dystrophin was restored to levels ranging from 3 to 90% and the muscle histology was improved
Primary open-angle glaucoma (POAG)	mouse	Mutations in the myocilin gene	Intraocular injection of the virus containing of SpCas9/sgRNA targeting the MYOC mutation (Y437H)	Lower IOP and further glaucomatous damage prevention
Retinal degeneration	mouse	Mutation in the <i>Nrl</i> gene	Delivery of the AAVvector cayyring CRISPR-Cas9 to postmitotic photoreceptors	The treatment substantially mproved rod survival and preserved cone function
Hypertrophic cardiomyopathy (HCM)	human embryos	Mutation in the <i>MYBPC3</i> gene	Microinjection of the recombinant Cas9 protein, sgRNA and ssODN DNA into the cytoplasm of pronuclear stage zygotes 18 h after fertilization	The heterozygous <i>MYBPC3</i> mutation was corrected without evidence of off-target mutations
Cardiovascular disease	mouse	Mutations in the subtilisin/kexin type 9 gene <i>Pcsk9</i>	Injection of the Ad vector (adenoviral vector) containing BE3 and a gRNA targeting <i>Pcsk9</i> codon W159 (Ad-BE3-Pcsk9)	The base-editing rate was 10%–15% and the indel rate was about 2%, leading to reduce the plasma <i>PCSK9</i> and cholesterol levels.
Hereditary tyrosinemia	mouse	Mutation in the fumarylacetoacetate hydrolase gene <i>Fah</i>	Injection of the Ad vector containing BE3 and a gRNA targeting the upstream <i>HPD</i> enzyme Q352 (Ad-BE3-Hpd)	The lethal phenotype of hereditary tyrosinemia type 1 was rescued.
Phenylketonuria	mouse	Point mutation in the <i>Pah</i> gene on exon 7 (c.835 T > C)	Injection of a dual AAV system and split nSaKKH-BE3 targeting <i>Pah^{enu2}</i> gene into the adult mice	The mutant of <i>Pah^{enu2}</i> gene was corrected, the phenylalanine hydroxylase enzyme activity was restored, and the light fur phenotype was reversed in <i>Pah^{enu2}</i> mice.
Human genetic deafness	mouse	A dominant-negative missense mutation in the <i>TMC1</i>	Injection of the Cas9–guide RNA–lipid complexes targeting the <i>Tmc1^{Bth}</i> allele into the cochlea of neonatal <i>Tmc1^{Bth/+}</i> mice	Higher hair cell survival rate and lower auditory brainstem response thresholds were observed
Cystic Fibrosis	organoids	Mutation of cystic fibrosis transmembrane conductor receptor (CFTR) locus	The mutant F508 del allele was corrected using the CRISPR/ Cas9 mediated homologous recombination	Functionality of the corrected allele in the organoid system was demonstrated
β-thalassemia	mouse	Either point mutations or deletions in the β-globin (<i>HBB</i>) gene	Generation of the iPSC-derived hematopoietic stem cells (HSCs) from the somatic cells of patients, correction of the mutations of <i>HBB</i> gene by CRISPR/Cas9 system	No observations of tumor formation in the mice model after the iPSCs transplantation
Microbial infection	mouse	Infection of bacteria with antibiotic resistance genes	Delivery of the RNA-guided nuclease Cas9 targeting antibiotic resistance genes by the bacteriophage	The bacteria in a mouse skin colonization model was killed successfully
Tumor	mouse	Mutiple pathegenetic mechanisms	Delivery of the CRISPR-based engineered CAR-T cells (such as PD-1 disrupted CAR-T cells) into the mice model	The efficiency of the engineered CAR-T cells is higher



STUDY CASES

Nothing is created

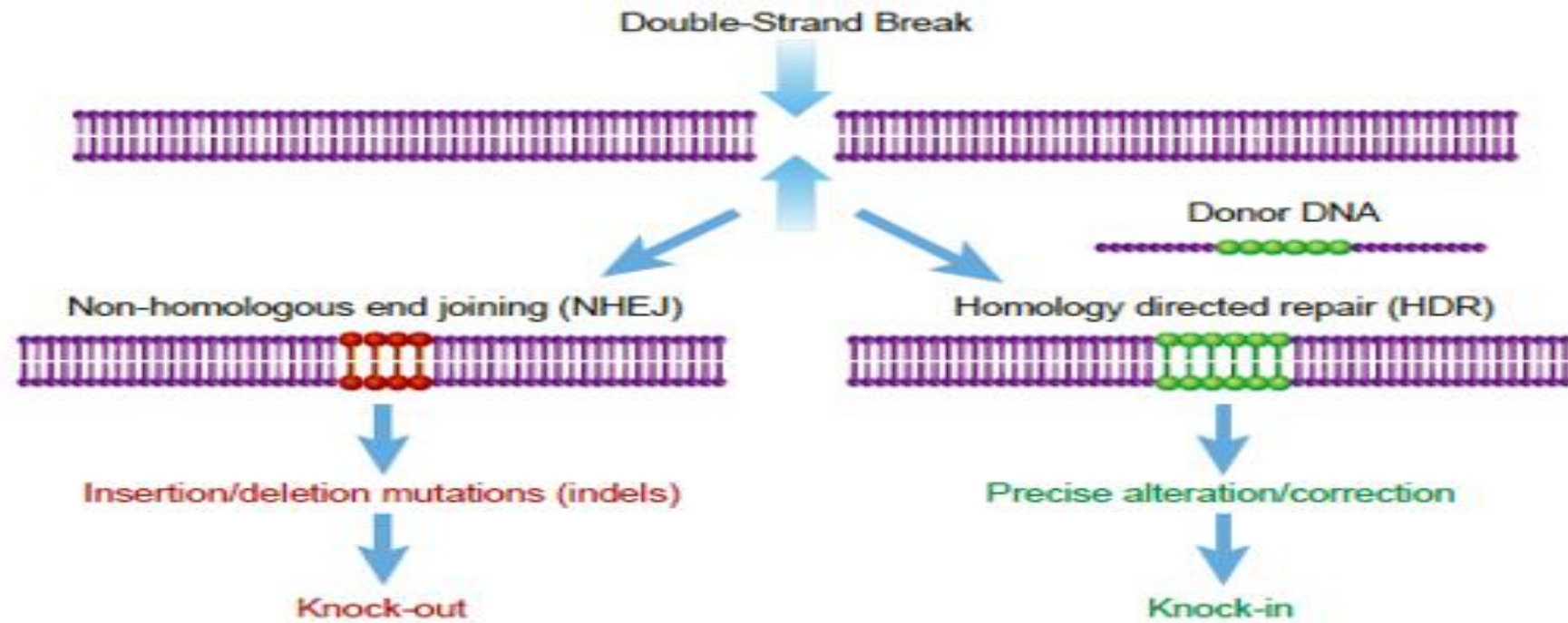
Nothing is destroyed

Everything is transformed

CRISPR GENETIC MODIFICATIONS

CRISPR - Cas9 works using knock-out and knock-in methods.

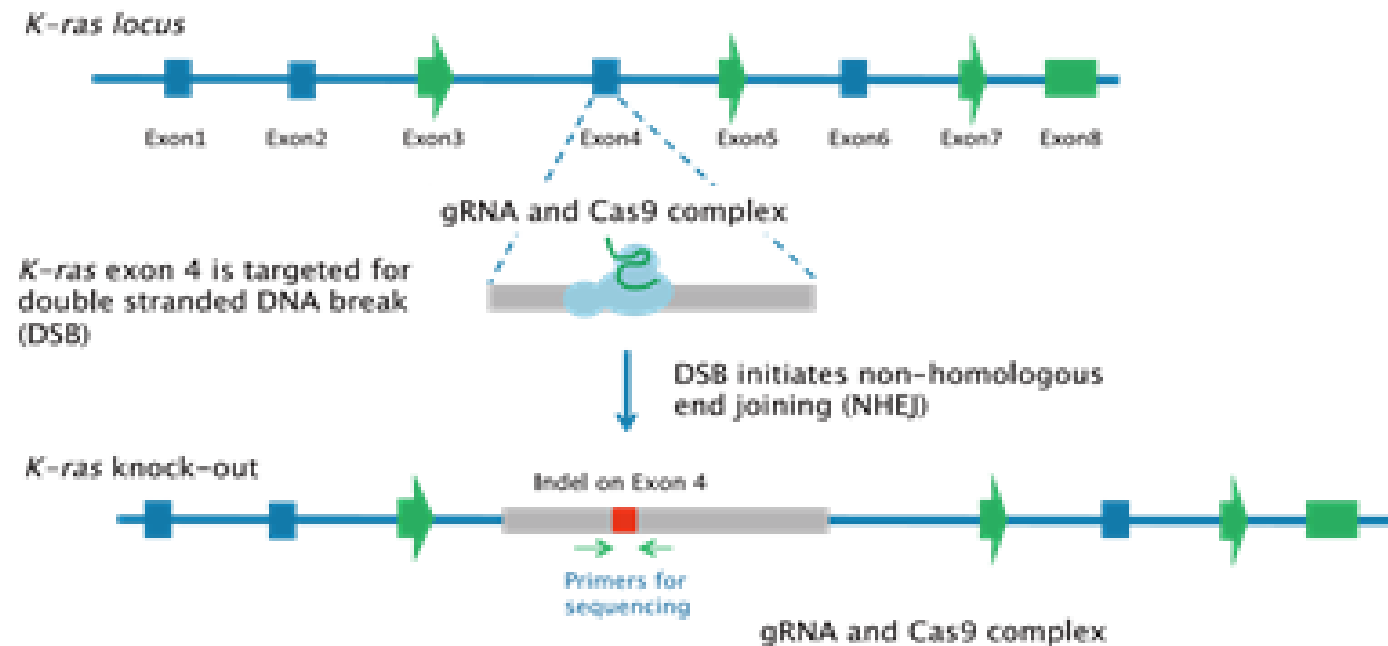
Genetic Modification	Application	Nuclease Activity	gRNA
Knock-out	Permanently remove gene function	Cas or Cas9n	gRNA targeting 5' exon or essential protein domains
Knock-in	Generate a specific sequence change	Cas or Cas9n	gRNA targeting region of interest
Interference	Reduce gene expression	dCas-repressor	gRNA targeting gene promoter elements
Activation	Increase gene expression	dCas-activator	gRNA targeting gene promoter elements



Knock-out method exploits the NHEJ technique (non-homologous end-joining)

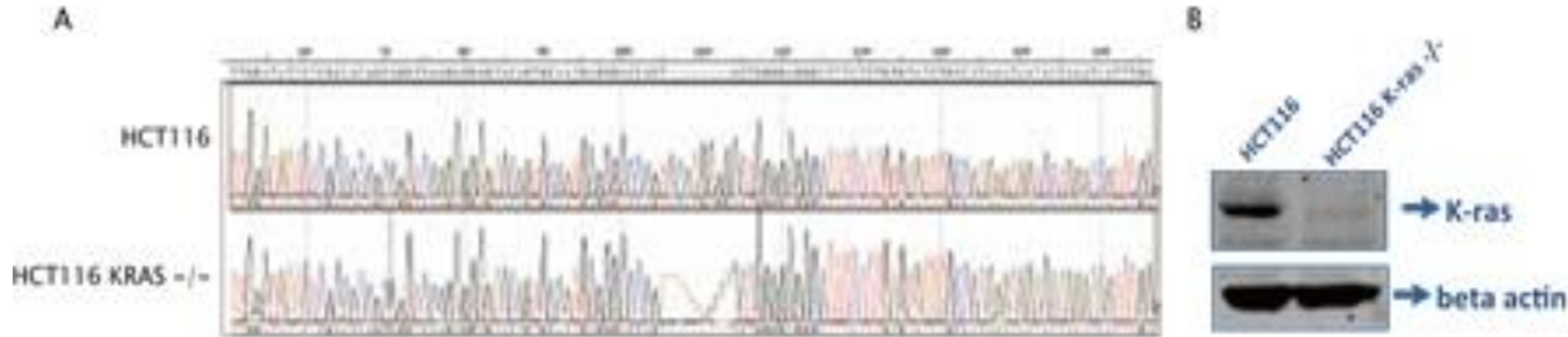
Knock-in method uses the HDR (homology directed repair) technique

GENERATION OF CELLULAR LINES TO DIRECT ELIMINATION



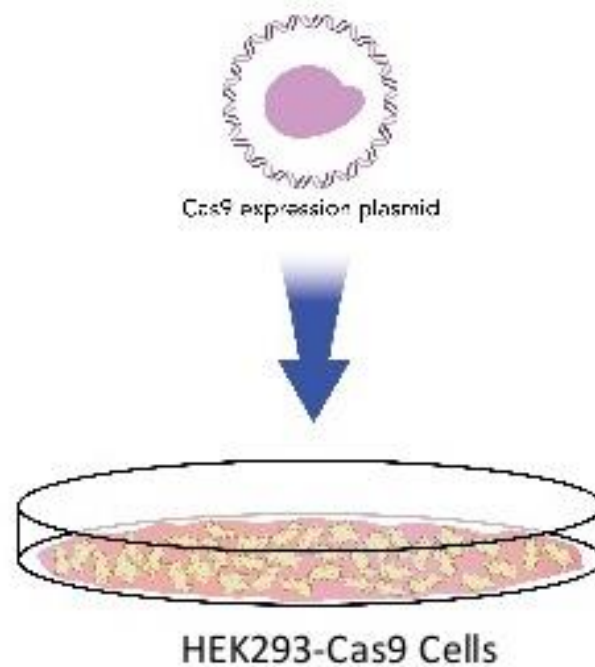
In this study case the researchers, using the knock out method, inactivated the KRAS gene, striking the exon 4 through the CRISPR-Cas9 mediator inserted using lentiviral vectors.

The figure represents the loss of the KRAS gene function

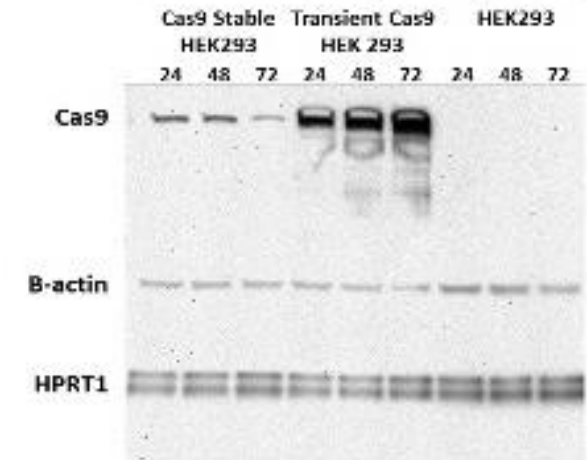


The Immunofixation or Western blotting method and Sanger sequencing are used to verify that the inactivation process has been successful.

KNOCK-OUT USING A POOL OF CELLS



Western blot— Cas9 primary antibody

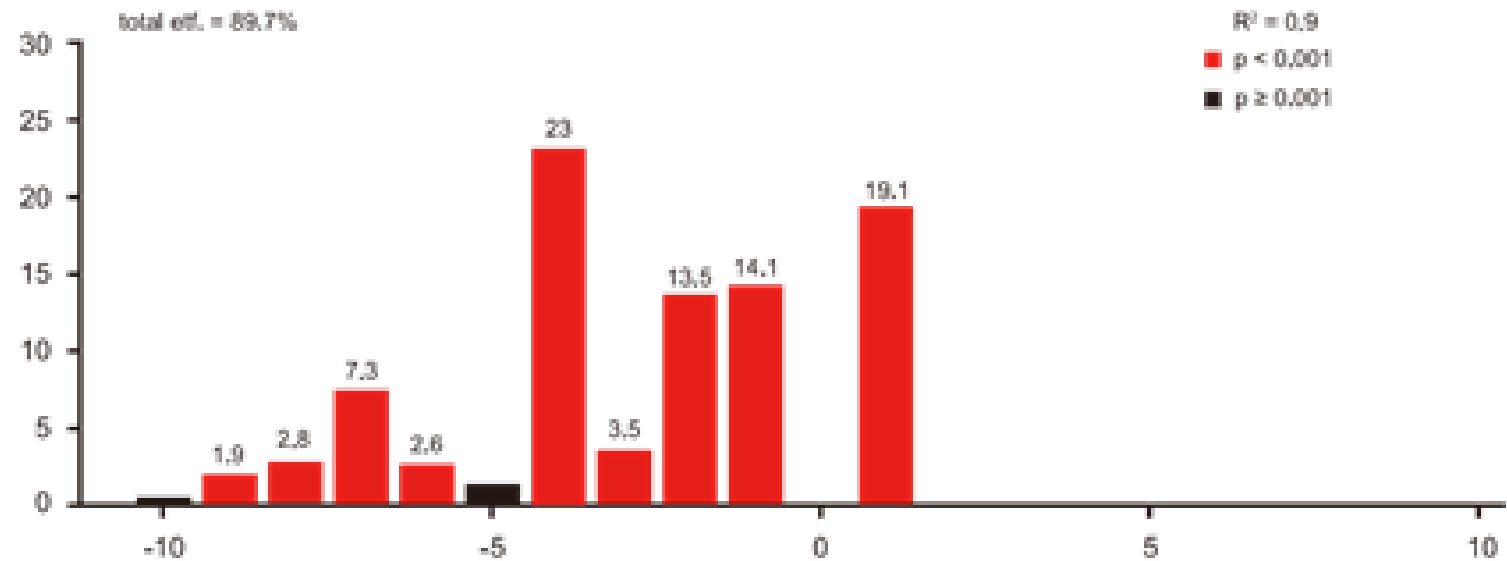


In this trial the researchers used the cell pool to attack the DNAJC3 gene in the HEK293 cell line, derived from human embryonic kidney cells.

CRISPR-Cas9 cell pools are heterogeneous cell cultures of stable cells in which Cas9 and gRNA are integrated in the genome.

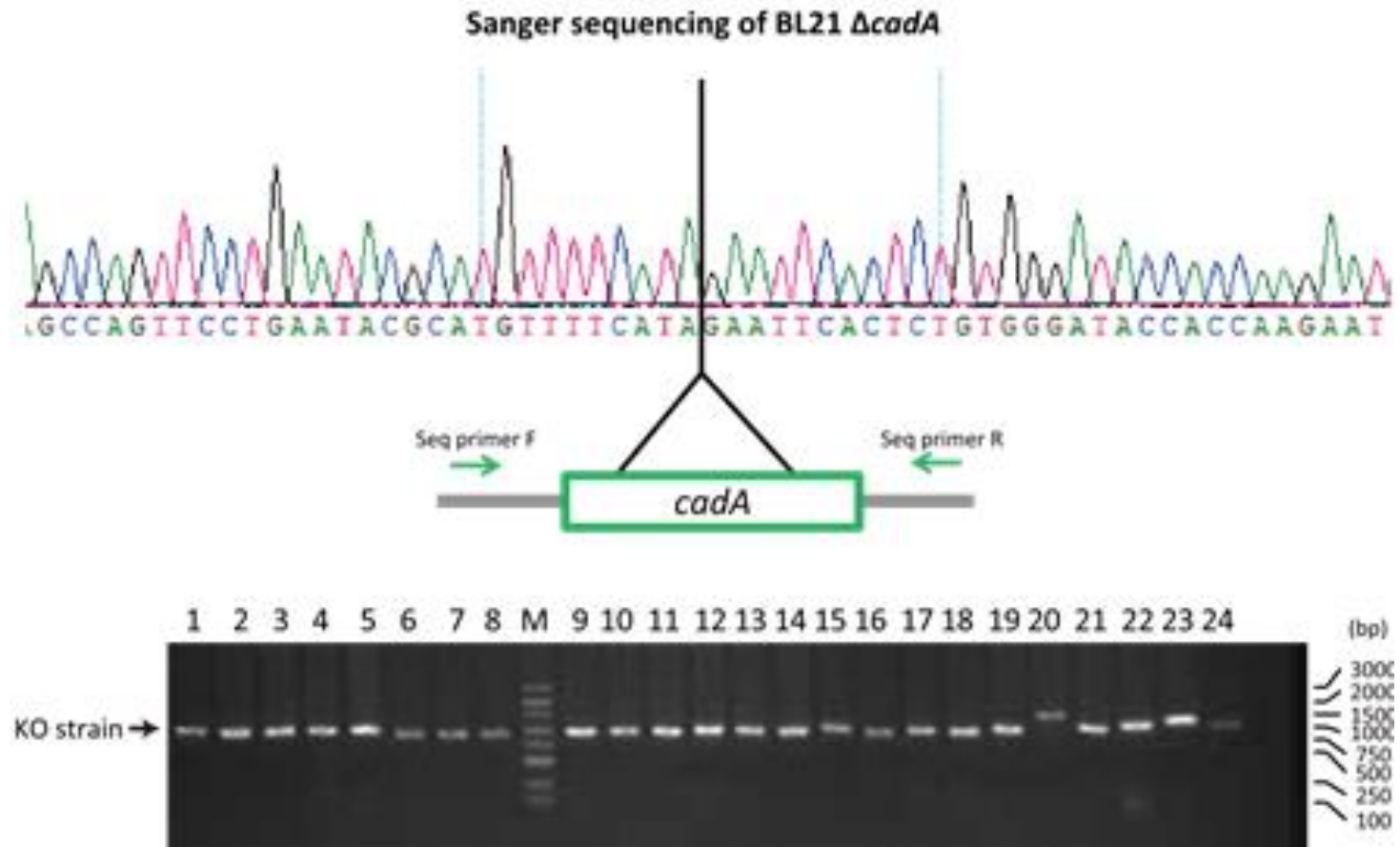
Efficiency of the indel mutation of the CRISPR DNAJC3 cell pool

The HEK 293 cells, after being transfected and selected based on antibiotic resistance, were analyzed by Sanger sequencing. It has been verified that the efficiency of the indel mutation is 89.7%.



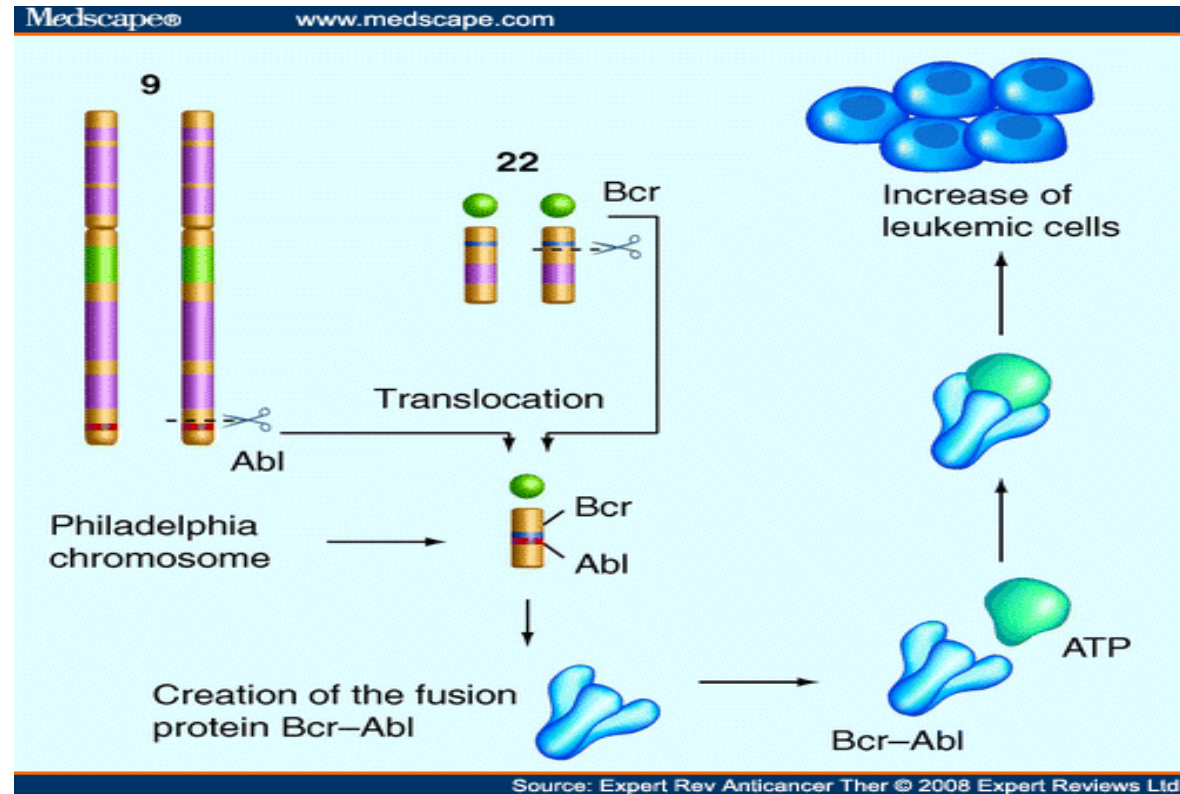
CRISPR FOR MICROBIAL GENETIC EDITING

The CRISPR-Cas9 technology can also be used in microbes by applying an optimization using the λ -Red technique. In this case, they used this technique to eliminate the *cadA* in the BL21 strain of *E. coli*. Using sequencing and Colony PRC screening they verified gene inactivation.



CHRONIC MIELOID LEUCEMY

In this research the CRISPR-Cas9 is used to attack the chimera gene BCR / ABL. The researchers found that by inhibiting this gene and therefore the synthesis of the p210 protein, the growth of tumor cells is reduced and their rate of apoptosis is increased.



CML is a myeloproliferative pathology that arises from the translocation of the ABL gene that binds to the BCR region of chromosome 22, creating the genetic chimera BCR / ABL responsible for the synthesis of the oncoprotein p210.

First phase: they tested a number of gRNA sequences in a cellular model of CML 3 to drive CRISPR-Cas9.



The result was favorable for all three cases.

Second phase: they moved to the in-vitro study to evaluate the effect during cell cycle progression.



The data show that it is actually possible to inhibit the gene and thus block the p210 protein by reducing the growth capacity of tumor cells and increasing the rate of apoptosis.

Third phase: in vivo study in a mouse model for 3 weeks, also using the Bcr-Abl-SC cell line.



The results demonstrate a considerable decrease in the mass of tumors. In particular, no tumor growth was observed using the Bcr-Abl-SC cell line.

GENE P53

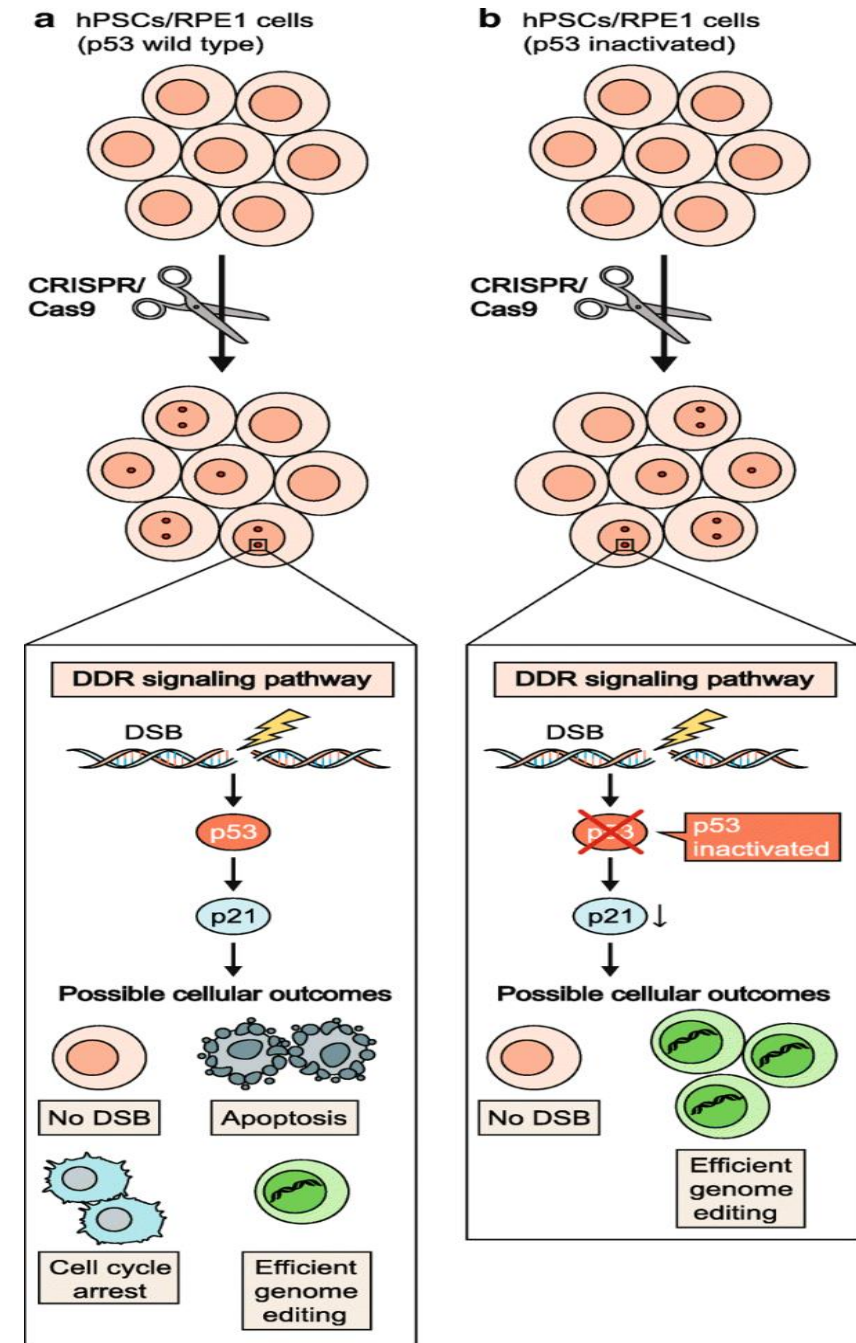


The research carried out simultaneously by two research groups, one Swedish and one Finnish on different cell populations, has led to very similar results.

- The former comparing cancer cells and retinal cells have observed that the action of CRISPR-Cas9 is much better in those of the tumor.
- The latter, on the other hand, experimenting the technique on pluripotent stem cells, saw that the cells themselves died.

These two phenomena are related to the activation of the p53 gene.

The p53 gene is called a tumor suppressor gene because it is responsible for the synthesis of the protein that triggers the "first aid" process to repair the damage caused by DNA or which triggers the apoptosis process if the damage is too important. These studies have therefore shown that to have a good efficiency the CRISPR-Cas9 method could select the cells that have the defective p53 gene.

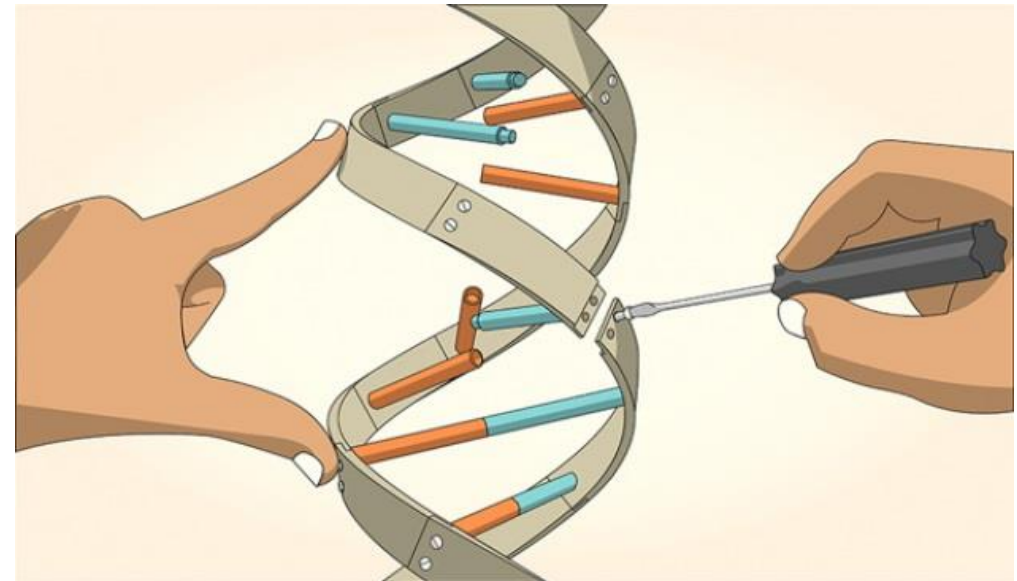


Future developments

If they're roses they'll bloom

Some comments

- CRISPR-Cas9 is rapidly reforming the biomedical industry.
- CRISPR-Cas9 has revolutionized genetic editing due to its ease of use and **applicability** to mammalian cells, microbes and animal models.
- CRISPR-Cas9 represents the best technology in terms of **cost-effectiveness**.
- CRISPR-Cas9 has the potential to increase our ability to **analyze** and **understand** genetic features.





EVERYWHERE

Illustration by Chris Labrooy ©nature

Some comments

- The structure of Cas9 and CRISPR will become **more and more known** and this will allow to create more efficient variants in order to open more and more ways to the CRISPR-Cas9 system.
- Undoubtedly, in the future, CRISPR-Cas9 technology will play a significant **role in the innovation** of research and industry.
- Even the general public will be more and more in touch with this technology, which has all the credentials for a **large-scale diffusion**.

New possibilities

01

Correction of
genetic
mutations

02

Control of
diseases or
hereditary
disorders

03

Large-scale
screening for
new drugs

04

New
generation
diagnostic
tests

05

Treatment of
aggressive
tumors

06

Immune
system editing



Some studies are
already under way

New possibilities

07

Applications in food

08

Applications in the environment

09

Animal-human transplants

20 people / day die waiting for an organ

10

Treatment of infectious diseases and antibiotic resistance

11

Possibility to modify cells by teaching them not to grow old

Promise of eternal life?

Ethical problem

Are they really all roses?

Everyone talks about CRISPR!



Crispr gene therapy fixes disease in a human embryo



ScienceNews



NEWS GENETICS, SCIENCE & SOCIETY

Researchers say CRISPR edits to a human embryo worked. But critics still doubt it

SCIENCE FOR THE CURIOUS
Discover

FROM THE JANUARY/FEBRUARY 2018 ISSUE

#5 Human Embryo Gets CRISPR Treatment

Researchers in a U.S. lab finally test the revolutionary gene-editing tool in human embryos.

MENU ▾

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Did CRISPR really fix a genetic mutation in these human embryos?

Researchers provide more evidence for their landmark claim that gene editing rid embryos of a disease mutation — but scientists are still arguing over the results.

NCBI

National Center for
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CRISPR: Established Editor of Human Embryos?

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The
Economist

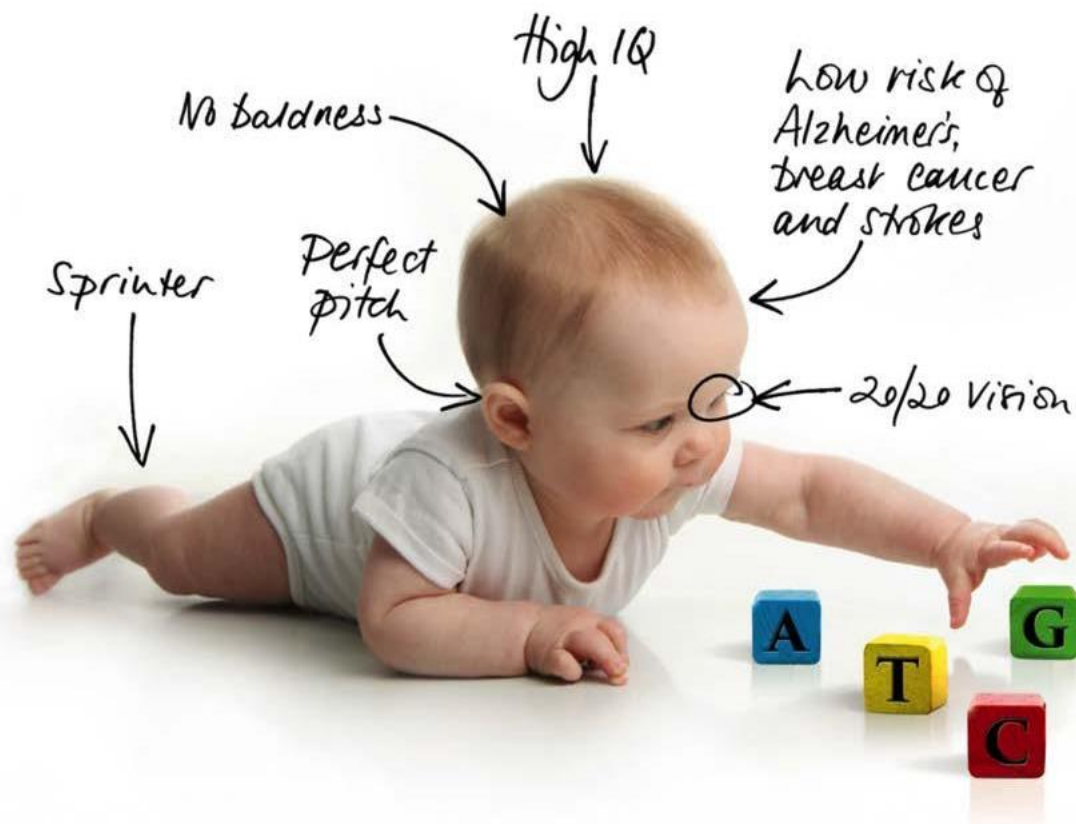
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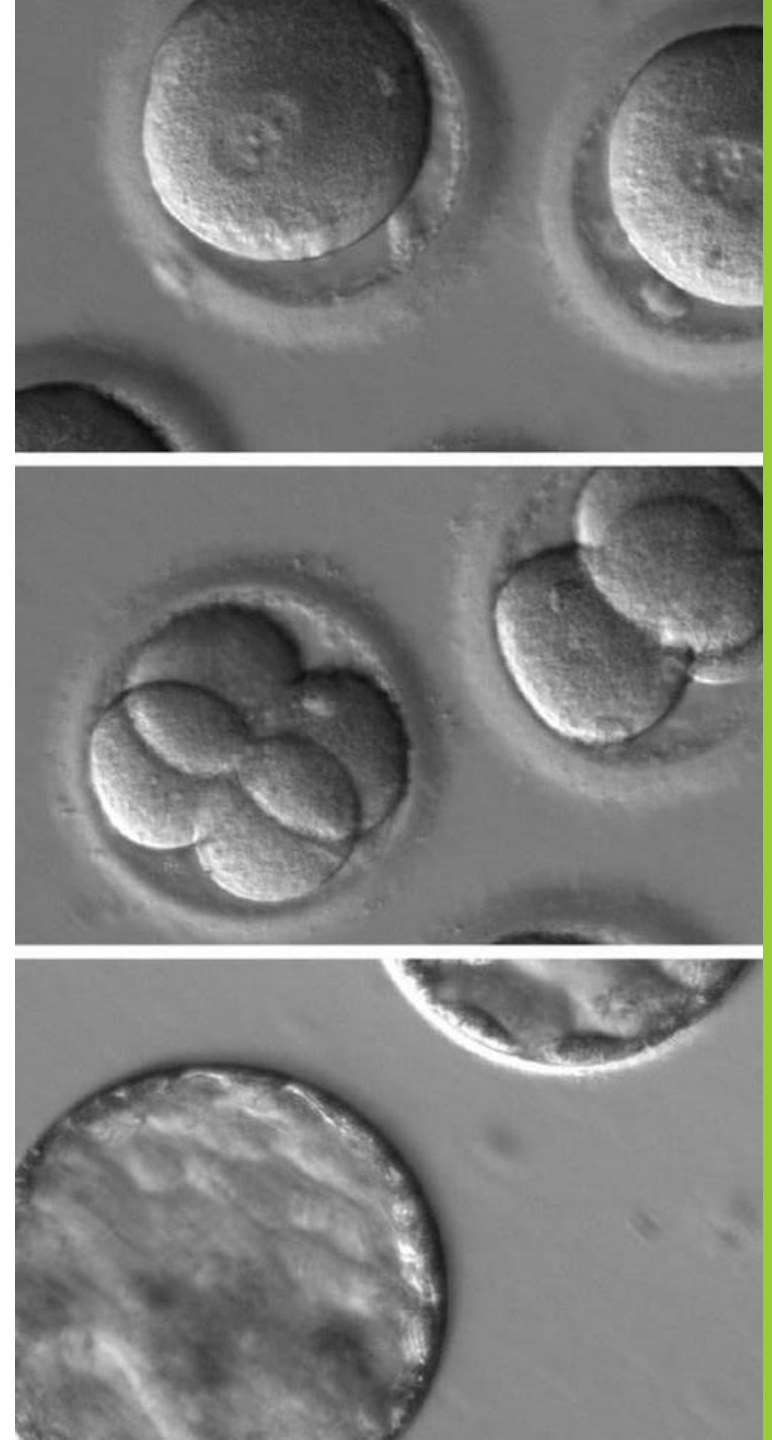
The inevitable fact

In 2015 a group of Chinese researchers applied the CRISPR-Cas9 technique to human embryos for the first time.

Obviously the news causes a sensation and gives rise to a new era where **perhaps**, one day, embryonic genetic modification will be a common practice.

The questions that arise are many:

- *When we talk about Baby Design, what is the line that divides an ethical change from an unethical one?*
- *In which areas is it correct to use CRISPR-Cas9? Only when it comes to health?*
- *Does it make sense to use CRISPR-Cas9 to reduce healthcare costs?*
- *Who will have access to the benefits and who will be excluded?*
- *Is it a safe technique?*





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Let's start from security

It is easy to consider that if the only parameter of evaluation of the ethicality of CRISPR-Cas9 was safety, then the obstacles would be surmountable.

But what is needed to achieve adequate levels of security?

Experiments and in-depth research on a large number of embryos with subsequent elimination of those in which there was a failure.

And is it ethical and / or correct to sacrifice human embryos?

Many fundamentalists would easily respond with a no, but is the answer really so simple?

Let's start from security



«If CRISPR-Cas9 could eliminate a mutation that causes an embryo disease with a high degree of certainty and only a small risk of introducing a second mutation off-target, the potential benefits could clearly outweigh the dangers.»
(Doudna)

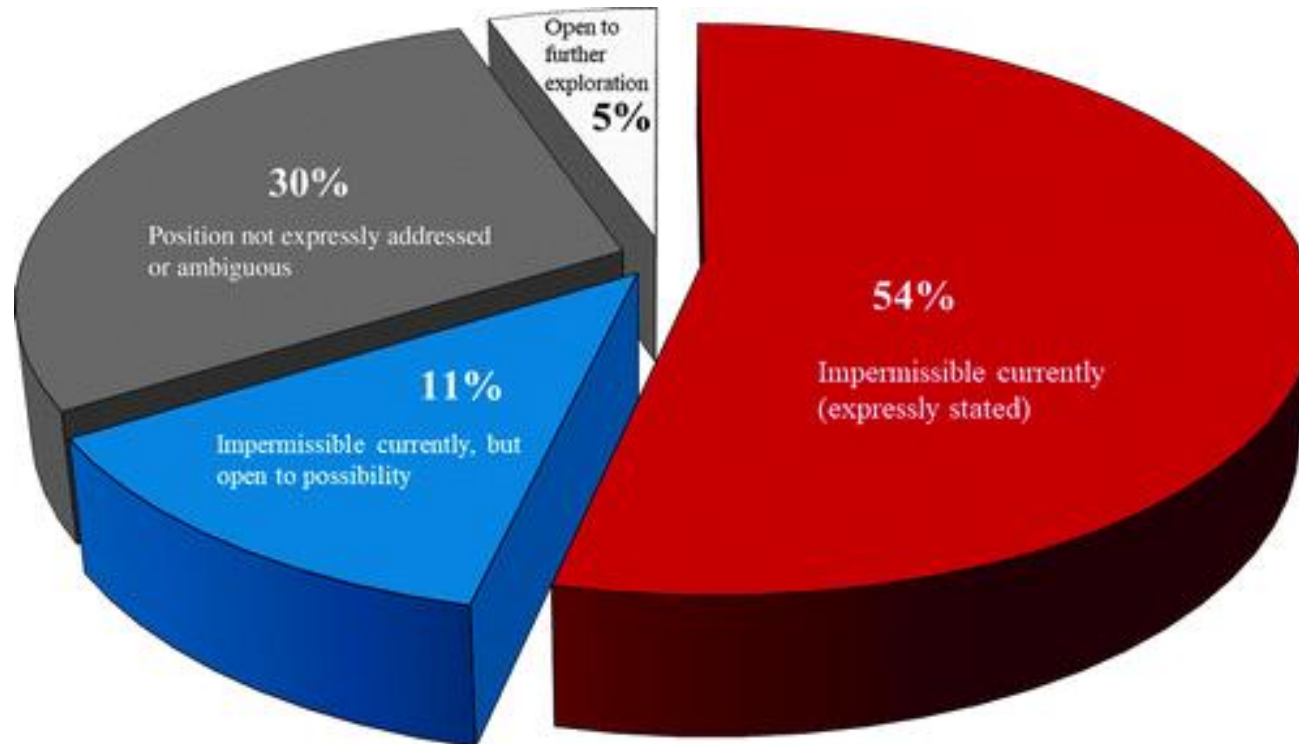
But a **single accident** what would it really represent?

It would be devastating for both the individual concerned and the whole industry, not to mention the wave of distrust of the general public towards this new technology.

*«I **fear** that the technology can be **abused** or represented in such a way as to damage the public perception of CRISPR-Cas9, confidence in its potential and acceptance of it as a tool for positive change.»* (Doudna)

The international community

In 2015 the International Summit on Human Gene Editing was born.



Carolyn Brokowski, 'Do CRISPR Germline Ethics Statements Cut It?', the CRISPR Journal.

The international community

Doudna proposes an attitude of **caution** and to draft **specific regulations**, regulations that are currently non-existent.

UNESCO declares its firm **opposition** to germinal editing, claiming that it would "renew eugenics".

"Interventions on the human genome should be admitted only for **preventive, diagnostic or therapeutic reasons** and without **enacting modifications for descendants**," says the IBC, arguing that the alternative would "jeopardize the inherent and therefore equal dignity of all human beings and renew eugenics."

(Adapted from 'Report of the IBC on Updating Its Reflection on the Human Genome and Human Rights')

The **researchers of the disability** are also divided in front of the dilemma of CRISPR, because if on the one hand there is the possibility of preventing diseases that cause enormous suffering, on the other there is the **fear** that with this step we will end up **rejecting the human diversity**.

Philosophers like Julian Savulescu argue that embryo editing would be an **ethical imperative**. In a world where people are dying of hunger, war, treatable diseases, raising the chances of guaranteeing healthy children to a small minority of people who have access to the most advanced techniques of embryology and genetics is legitimate , indeed good and right.



Latest reflections

*"Evolution has worked to optimize the human genome for **3.85 billion years**. Do we really think that a small group of human genome repairers can do better without any kind of unwanted consequences?"* (Francis Collins, former head of the international human DNA sequencing project and now heads the National Institutes of Health)

Probably in the future we will realize that by saying yes to CRISPR in the field of human reproduction we have **not opened the Pandora's box** nor freed the world from all evil.

As the Broad Institute director Eric Lander explained at the 2015 Summit, there will not be many people to take advantage of the germline application of this technique, which is already proving very useful to study the molecular basis of diseases, looking for new therapeutic approaches, and that promises to broaden the horizons of classical gene therapy, by **correcting the diseased cells** of the **individual patient's body**.

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Thank You!

Sabina Maglio, Giacomo Ciaperoni, Amedeo Bonatti, Andrea Vogliardi, Alessandro Lazzaro