

CRISPR/Cas9 for advanced DNA and RNA editing



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CRISPR/Cas9

**Paradigm
shift**

Nobel Prize



Holy Grail of GE

Disruptive Technology

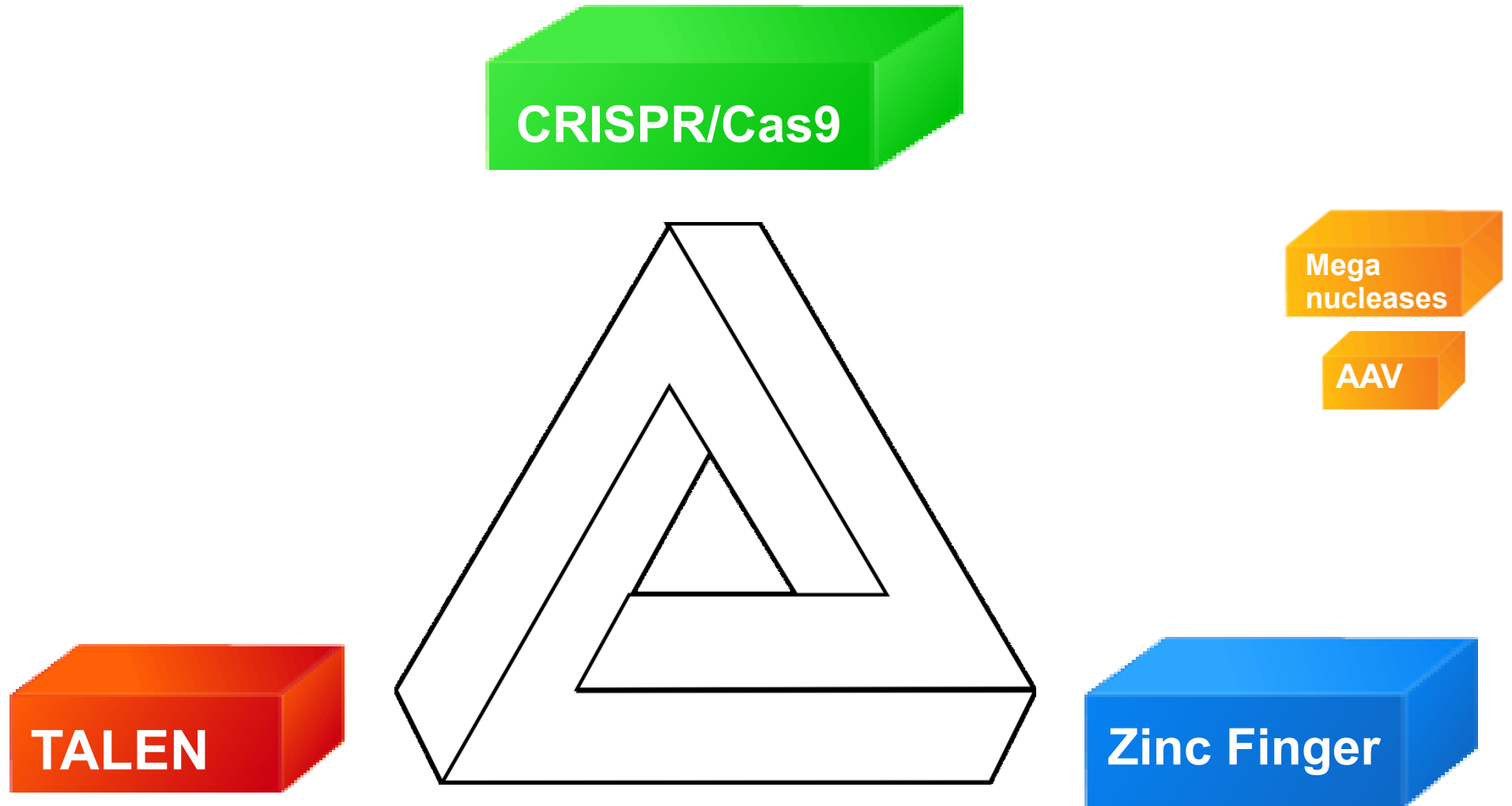


**Or The Emperor's
New Clothes?**

Juggernaut



The triumvirate of genome editing



Why is CRISPR/Cas9 better?

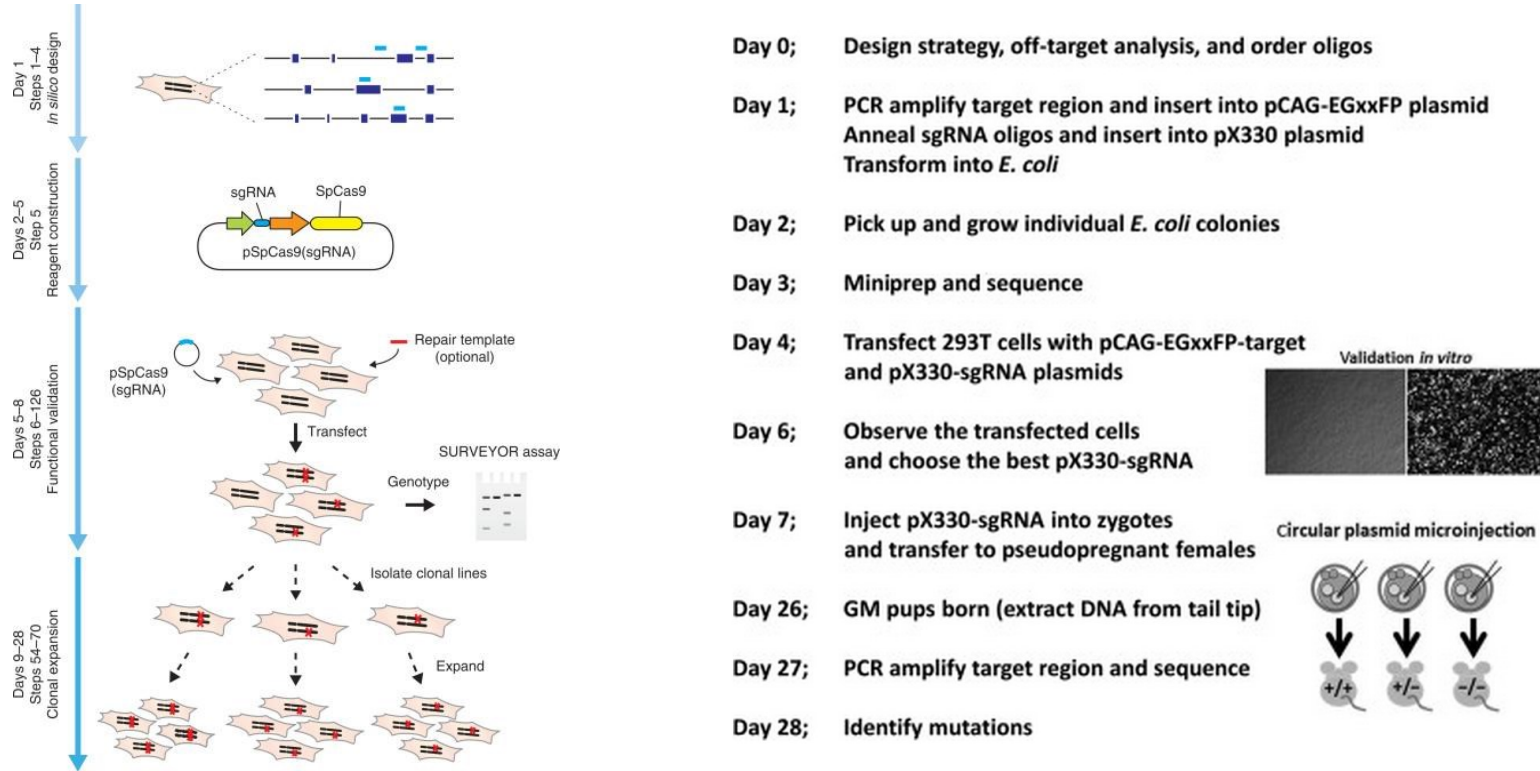
SPEED/Price



Simplicity

Accuracy/Specificity

CRISPR/Cas9 speed generation of transgenic mice

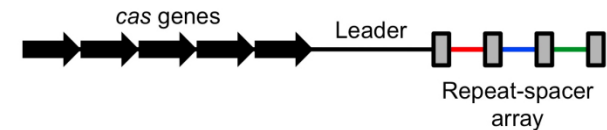



6 month (conventional)

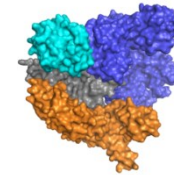

1 month (CRISPR/Cas9)

CRISPR loci and Cas nuclease nomenclature

CRISPR: Clustered Regularly Interspaced Palindromic Repeats
Loci in 40% of bacteria and 90% of archaea

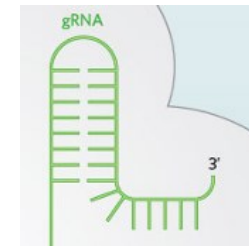


Cas9: CRISPR associated protein 9
a nuclease, an enzyme specialized for cutting DNA
Cas1..Cas10 exist

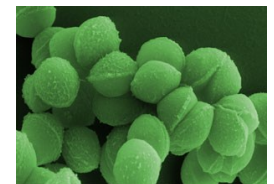


CRISPR/CAS: type I, type II and type III

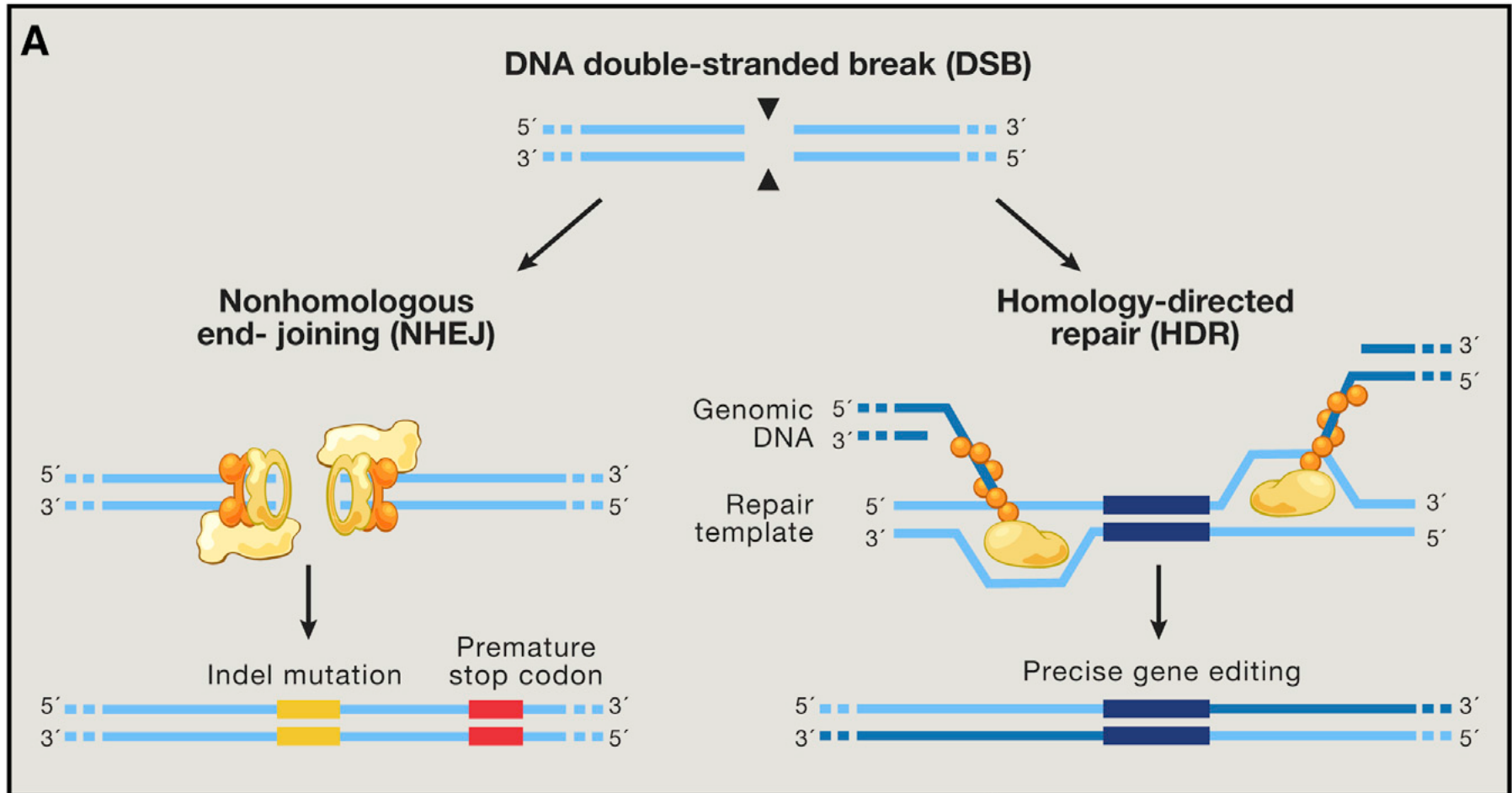
gRNA: guide RNA – a construct/chimera of CRISPR RNA (crRNA)
and trans-activating CRISPR RNA (tracrRNA)



PAM: protospacer adjacent motif with sequence
NGG (any, guanine, guanine) specific to *Streptococcus pyogenes*
and 5'-NAG (any, adenine, guanine) PAM tolerated in human cells



Endogenous DNA repair mechanisms



VIDEO: Genome Editing with CRISPR-Cas9



Click to watch

CRISPR/Cas9 process includes a targeted double strand break

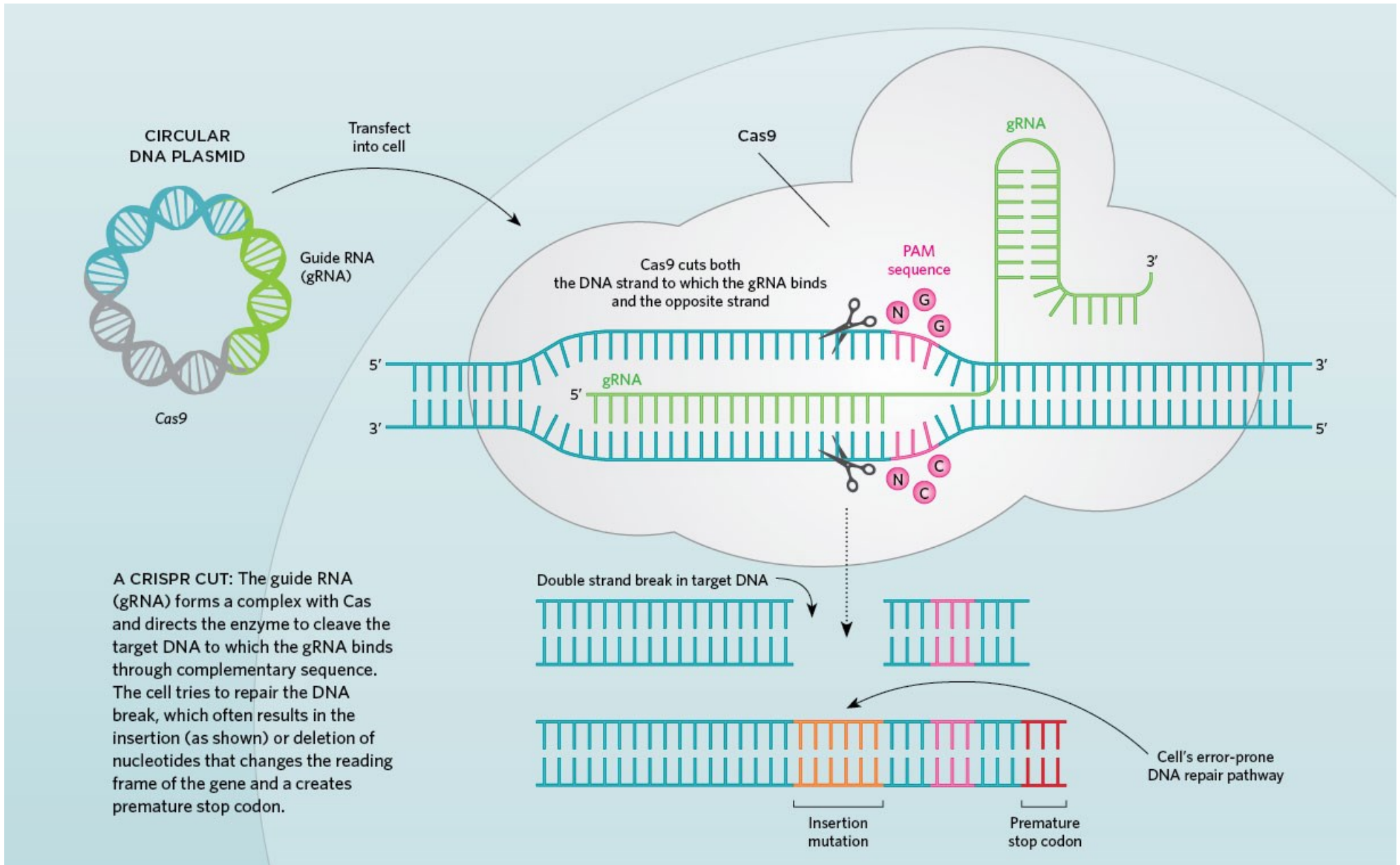
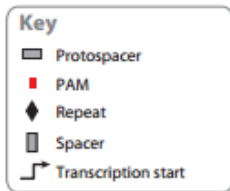
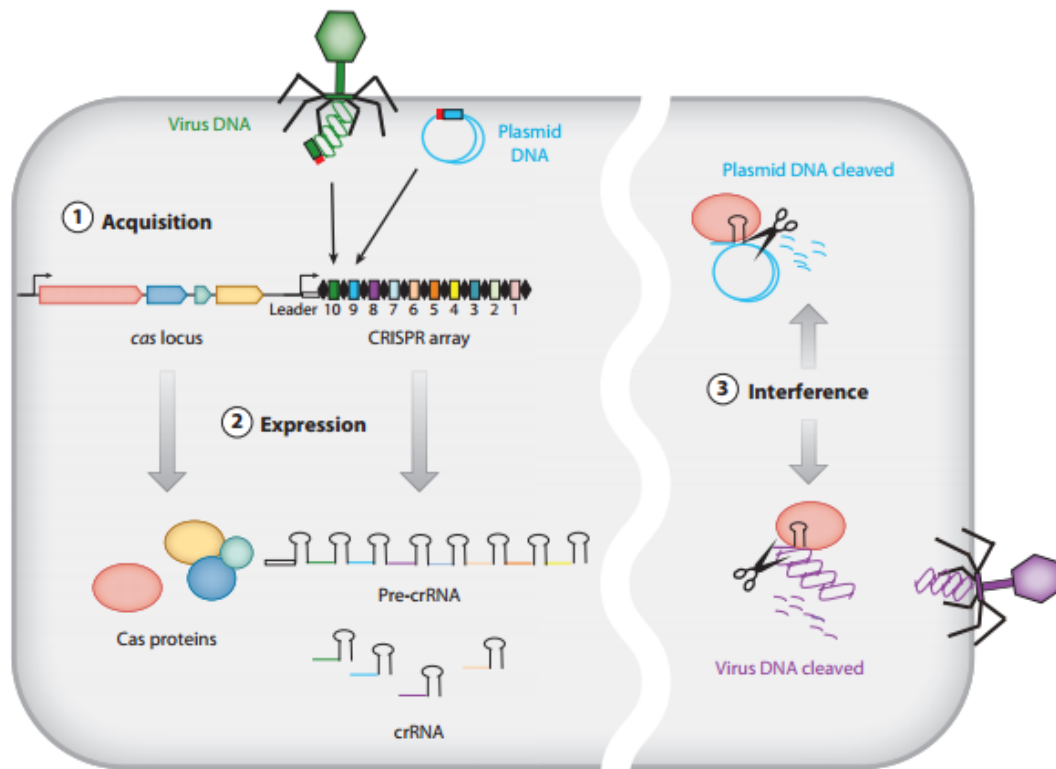
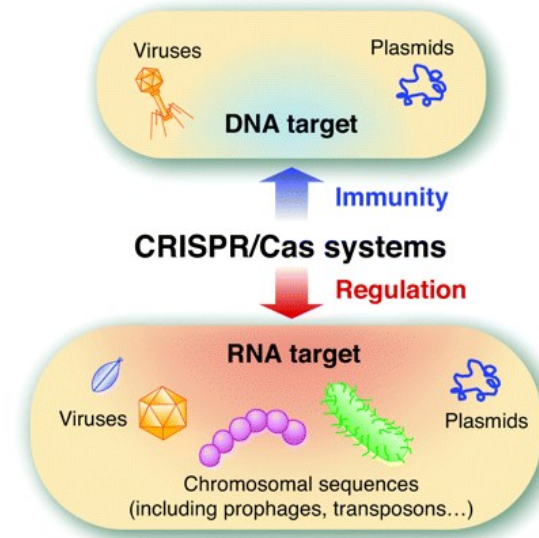


Figure: Storrs; A CRISPR Fore-Cas-t; (2014) The Scientist magazine; <http://www.the-scientist.com/?articles.view/articleNo/39239/title/A-CRISPR-Fore-Cas-t/>

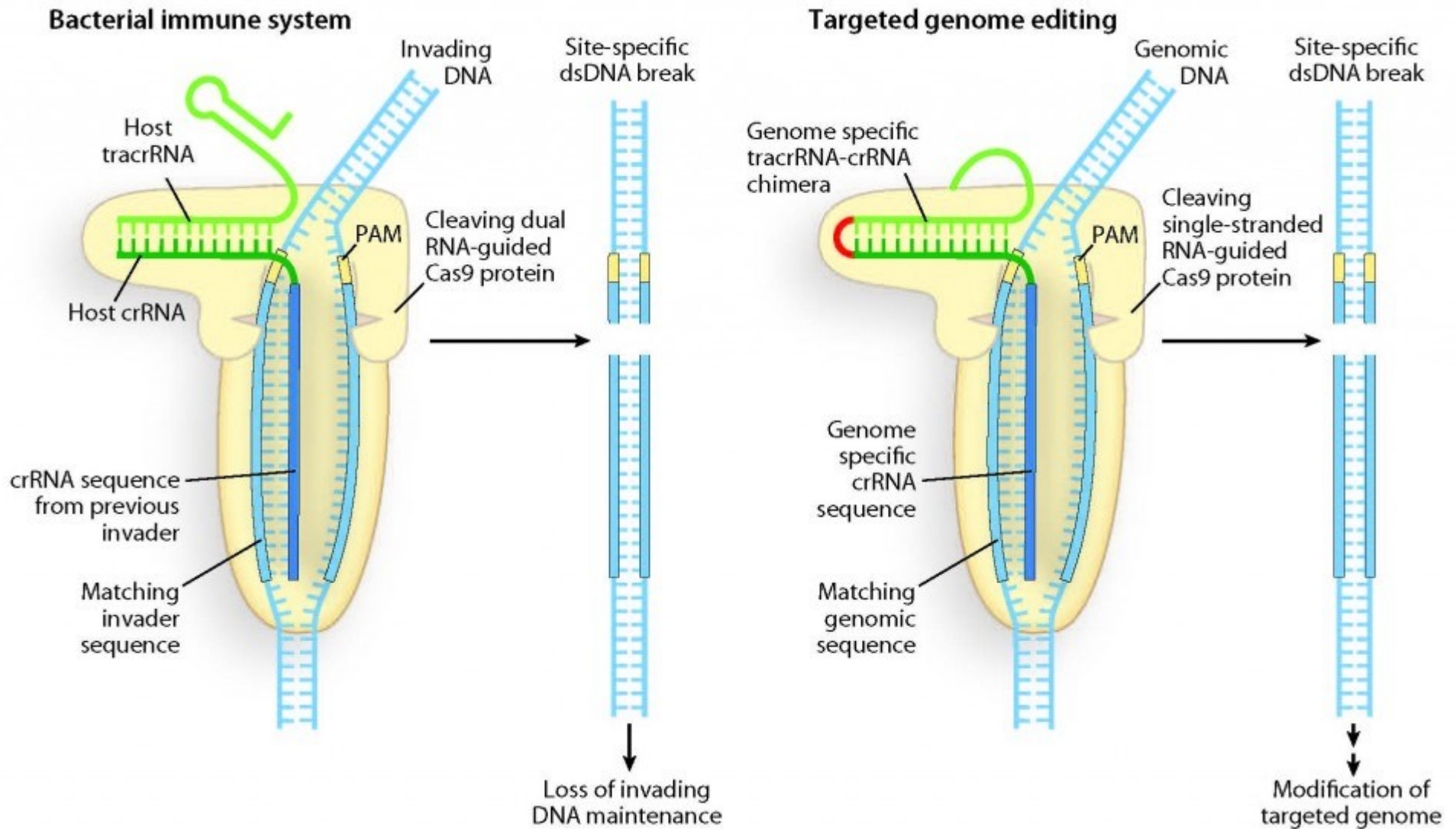
CRISPR/Cas the immune system of bacteria



- (1) acquisition of foreign DNA
- (2) synthesis and maturation of CRISPR RNA (crRNA) followed by formation of RNA-Cas nuclease protein complexes
- (3) target recognition by crRNA and destruction of foreign DNA by Cas nuclease cleavage

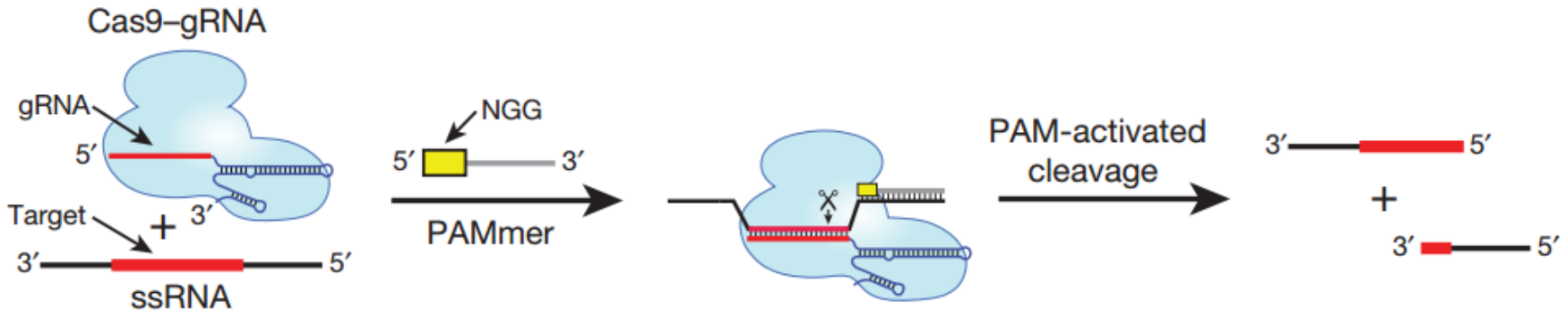


Programmable DNA scissors



Source: lbl.gov; Programmable DNA scissors: A double-RNA structure in the bacterial immune system has been discovered that directs Cas9 protein to cleave and destroy invading DNA at specific nucleotide sequences. This same dual RNA structure should be programmable for genome editing. (Image by H. Adam Steinberg, artforscience.com)

RNA editing with CRISPR/Cas9 using RCas9



RNA-guided Cas9 cleaves single stranded RNA (ssRNA) targets in the presence of a short PAMpresenting DNA oligonucleotide (PAMmer)

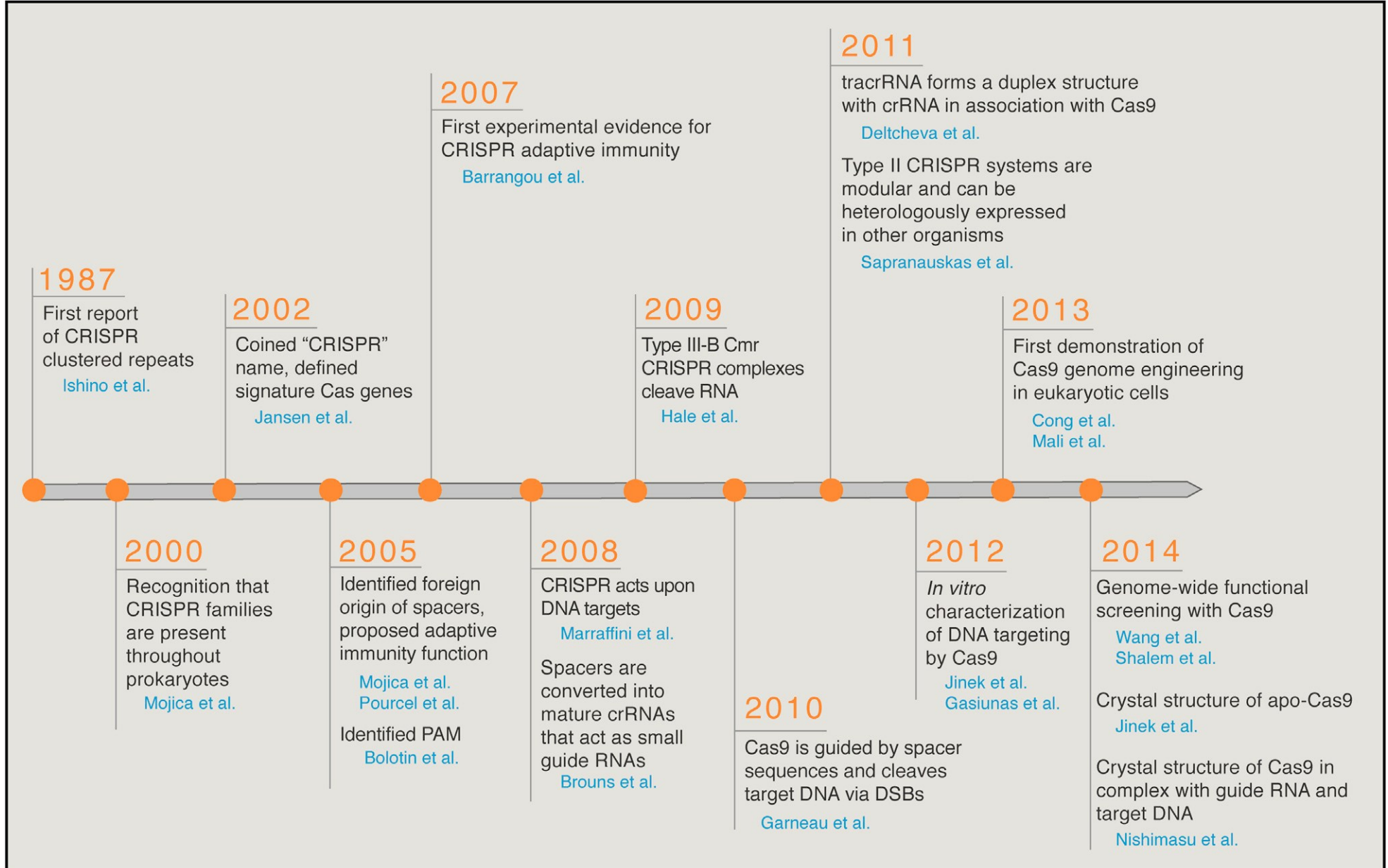
Requires only synthesis of a matching gRNA and complementary PAMmer and does not require affinity tags

Who are the actors in the CRISPR/Cas9 patent war?



***** Patents, Business, Legacy**

Timeline



Main Actors in CRISPR/CAS9 patent war*



Feng Zhang
(MIT/Broad)



Jennifer Doudna
(Berkeley/HHMI)



Emmanuelle Charpentier
(Helmholtz/MIMS/MHH)

Read: Antonio Regalado @ Technologyreview.com: Who Owns the Biggest Biotech Discovery of the Century?

(*) Ignores many other researchers and inventors, such as George Church (Harvard), Philippe Horvath, Rodolphe Barrangou (Danisco/Dupont)

Photo sources: Investigator websites

WIPO Patents for CRISPR 2015

by country, applicant and inventor

Countries	
Name	No
PCT	140
United States	71
China	29
European Patent Office	9
Russian Federation	8
Canada	6
Japan	4
Portugal	1
Mexico	1
Spain	1
EAPO	1

Main Applicant	
Name	No
THE BROAD INSTITUTE, INC.	14
SANGAMO BIOSCIENCES, INC.	11
CELLECTIS	11
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	9
PRESIDENT AND FELLOWS OF HARVARD COLLEGE	9
THE BROAD INSTITUTE INC.	8
Sangamo BioSciences, Inc.	7
The Broad Institute, Inc.	6
DOW AGROSCIENCES LLC	6
DANISCO	6

Main Inventor	
Name	No
ZHANG, Feng	18
Zhang Feng	11
ZHANG Feng	6
Barrangou Rodolphe	6
HORVATH PHILIPPE	5
GREGORY, Philip D.	4
DUCHATEAU, Philippe	4
Cost Gregory J.	4
CHURCH, George M.	4
BARRANGOU RODOLPHE	3

Rows are not related

Companies



Broad LIC



Broad LIC



Broad LIC



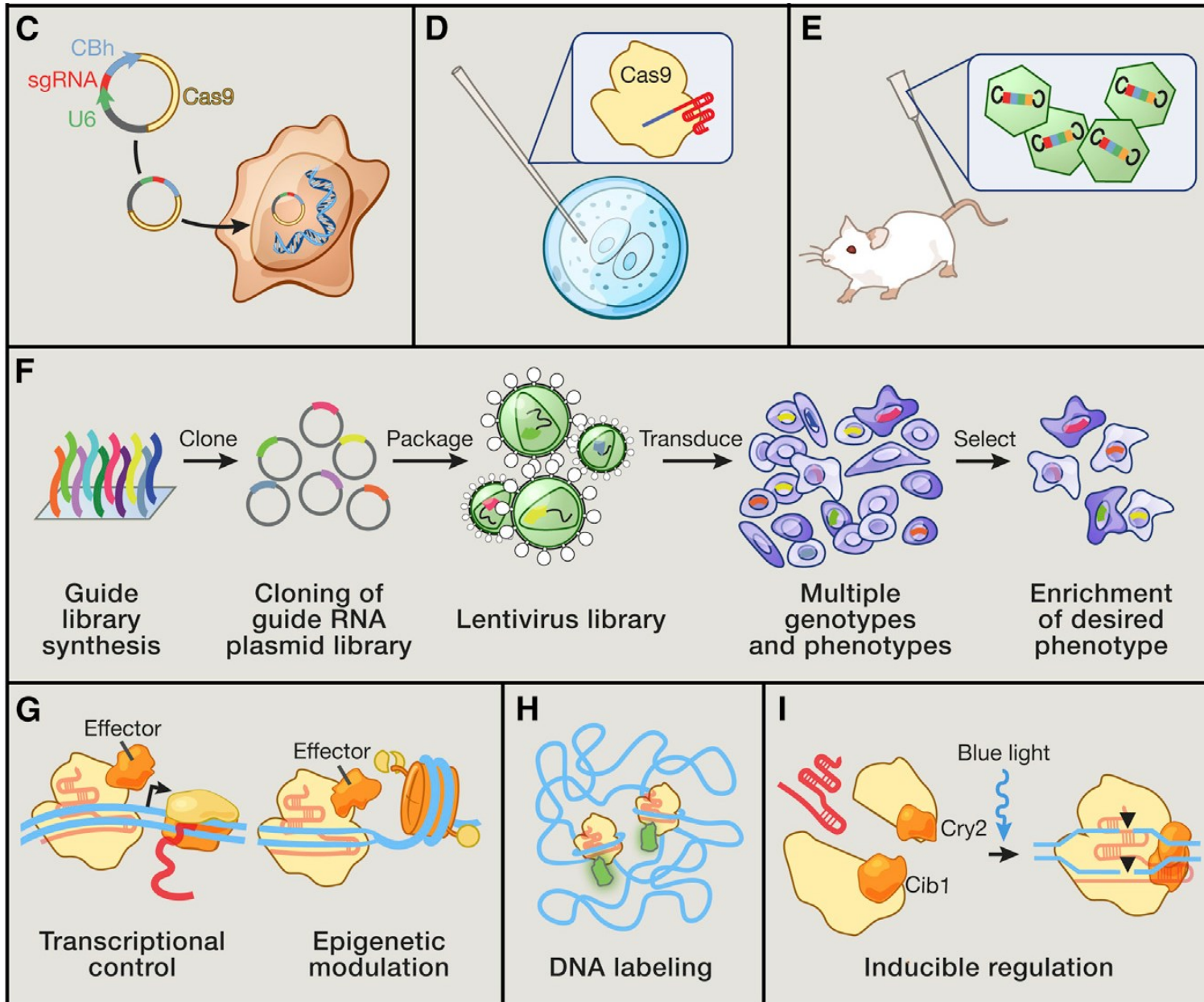
Broad, Caribou LIC



Broad LIC

Harvard, Broad, ERS LIC

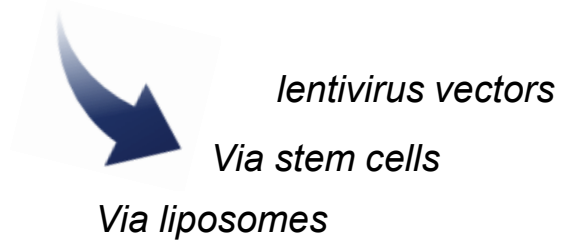
Application examples



Problems, problems, problems

Off-target effects and unintended DNA cleavage

Delivery to cells in humans

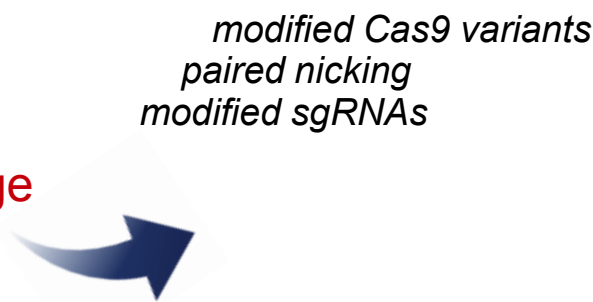


paired nickses
(single-strand DNA break)
double-nicking

Check with whole genome sequencing



Variable DNA cleavage efficiencies



Use DBS and Cas9 to correct

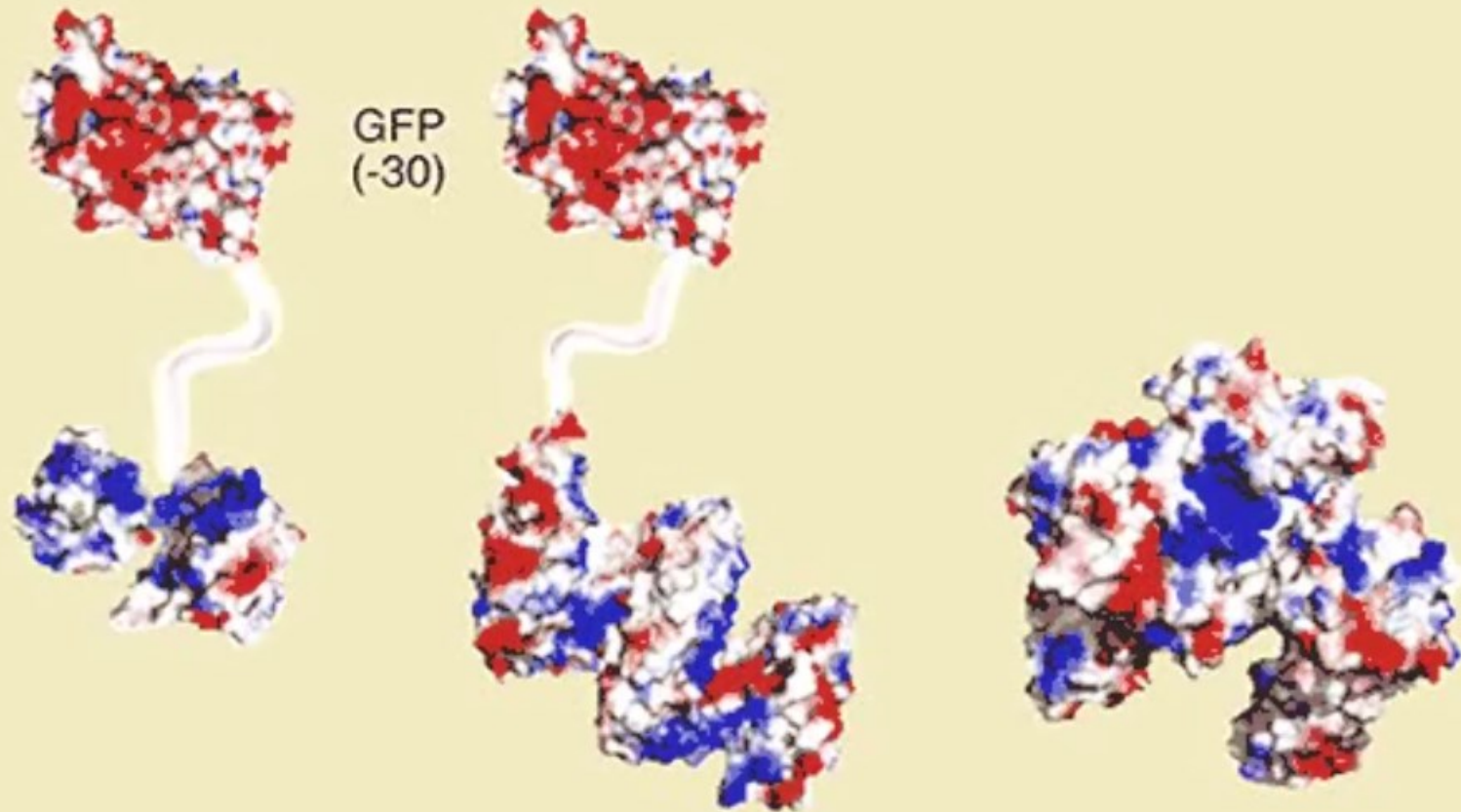


inter- and intrachromosomal rearrangement

Liposome delivery method

Stage 1: Engineer Negatively Charged Proteins

To impart a high degree of negative charge, a negatively “supercharged” molecule is associated with the genome-editing proteins.



Cre recombinase (+11)

Tale protein (+4)

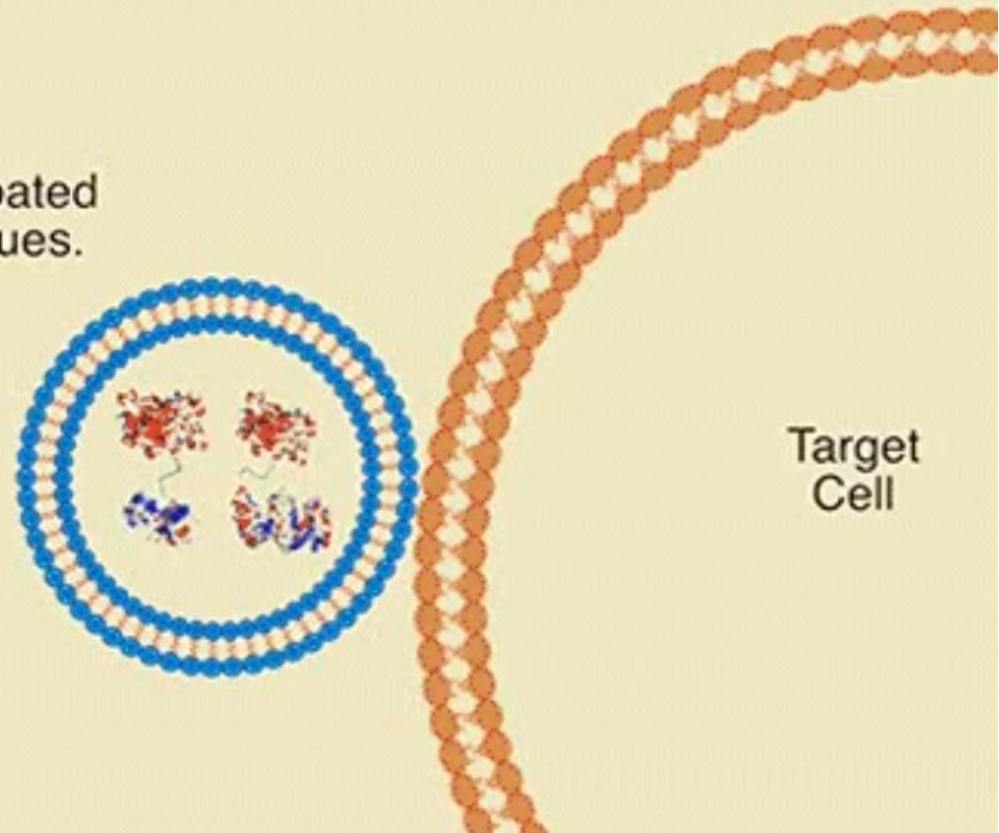
Cas9 (+22)

Liposome delivery method

Stage 2: Form Lipid Complexes

To deliver the highly negatively charged genome-editing proteins into cells, researchers complex them with cationic (positively charged) lipid molecules to form structures that include small vesicles called liposomes.

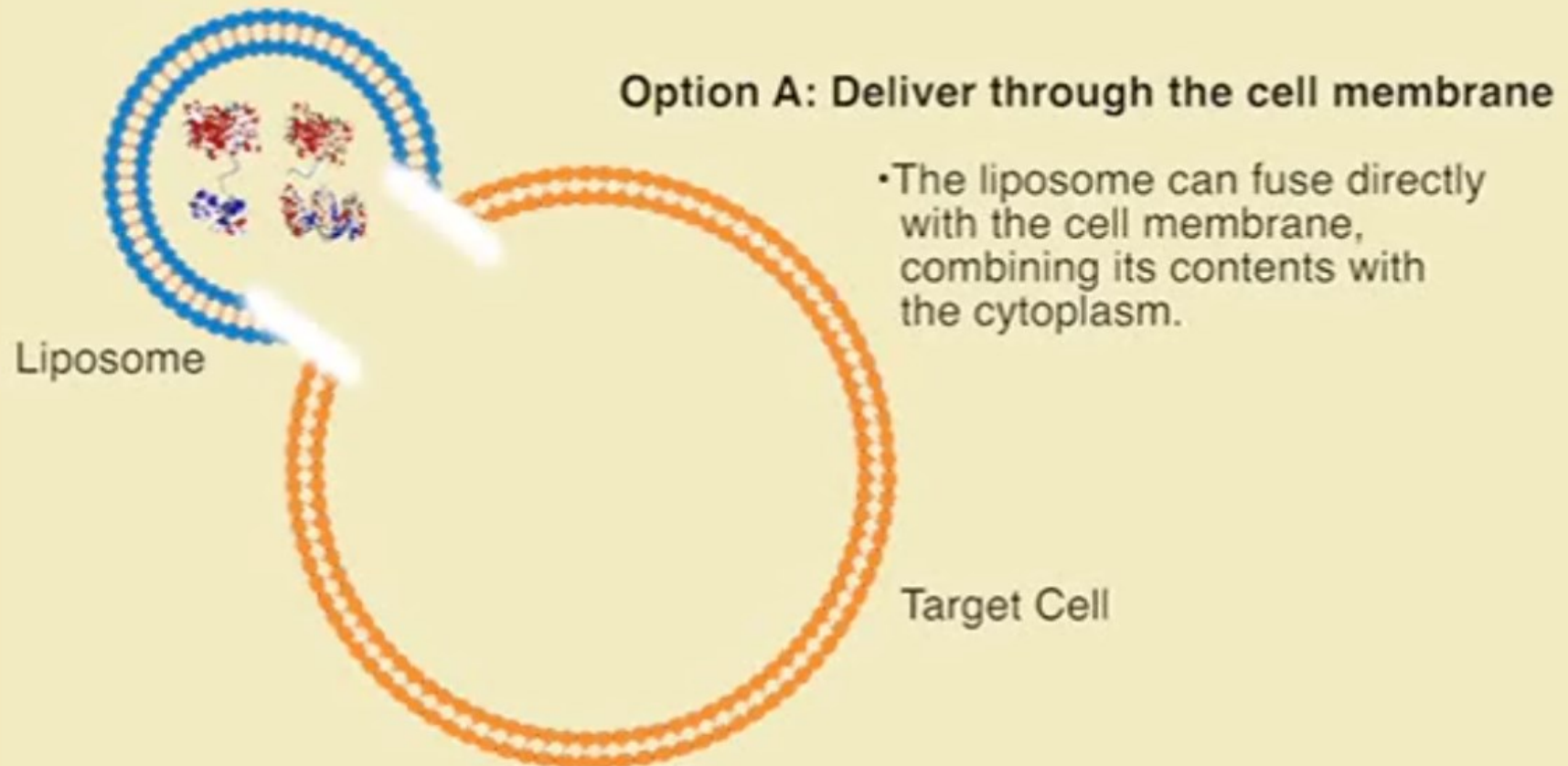
The liposomes are then incubated with cells or injected into tissues.



Liposome delivery method

Stage 3: Deliver into Cells

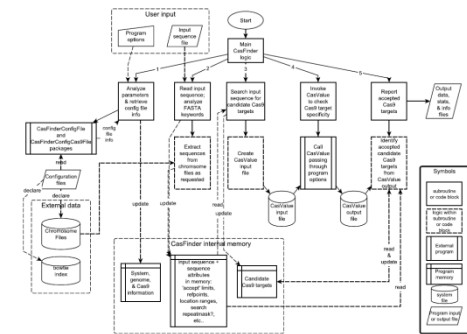
The genome-editing proteins can enter the cytoplasm of the cell through at least two mechanisms:



Software and databases



CasFinder



Rational design of CRISPR/Cas target.

ZiFiT Targeter

E-CRISP

Design of CRISPR constructs



Companies offering CRISPR/Cas9 services

TALEN and CRISPR services
www.genecopoeia.com/genome-editing ▾
Start from \$150 Quick delivery!
Validation, donor, cell line optional

CRISPR Mouse Models
www.genetargeting.com/CRISPR-Cas9 ▾
Includes conditional KOs & KIs
CRISPR model development guaranteed

CRISPR Reagents
www.sageresearchlabs.com/ ▾
Validated **CRISPRs**
Custom **designed** for your project

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www.scienceexchange.com/CRISPR ▾
(855) 467-2439
CRISPR and **Cas9** Genome Editing.
From Top, Verified Facilities

Inducible CRISPR Options
www.transomic.com/transEDIT-CRISPR ▾
All in one gRNA and Inducible **Cas9**
expression for regulatable editing

CRISPR/Cas9 Mouse Models
www.genoway.com/Nuclease-CRISPR ▾
Cost Effective & Fast Development.
Off-target effects assessment.

CRISPR-Cas9 Gene Knockout
www.collecta.com/ ▾
Stable & Permanent Knockout
All-in-One Lentiviral Vectors

CRISPR/Cas9 Antibody
www.diagenode.com/Cas9-antibody ▾
Excellent results in all major
applications - Immunoblot, IP, IF

CRISPR/Cas9 system
www.clontech.com/CRISPR ▾
Kits for successful **CRISPR/Cas9**
genome editing. Learn more

TALEN and CRISPR services
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Start from \$150 Quick delivery!
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Crispr
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www.scienceexchange.com/CRISPR ▾
CRISPR and **Cas9** Genome Editing.
Successful Results From Top Labs

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CRISPR model development guaranteed

CRISPR Reagents
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Validated **CRISPRs**
Custom designed for your project

SIGMA-ALDRICH

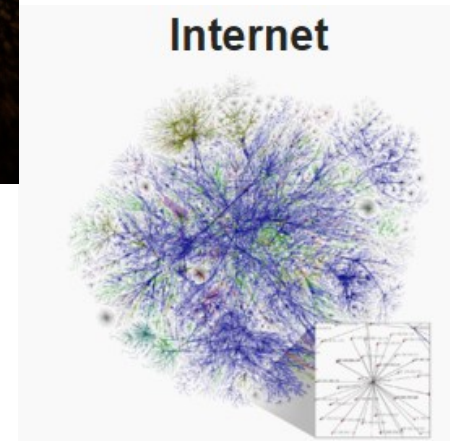
life
technologies

 GE Healthcare
Dharmacon RNAi

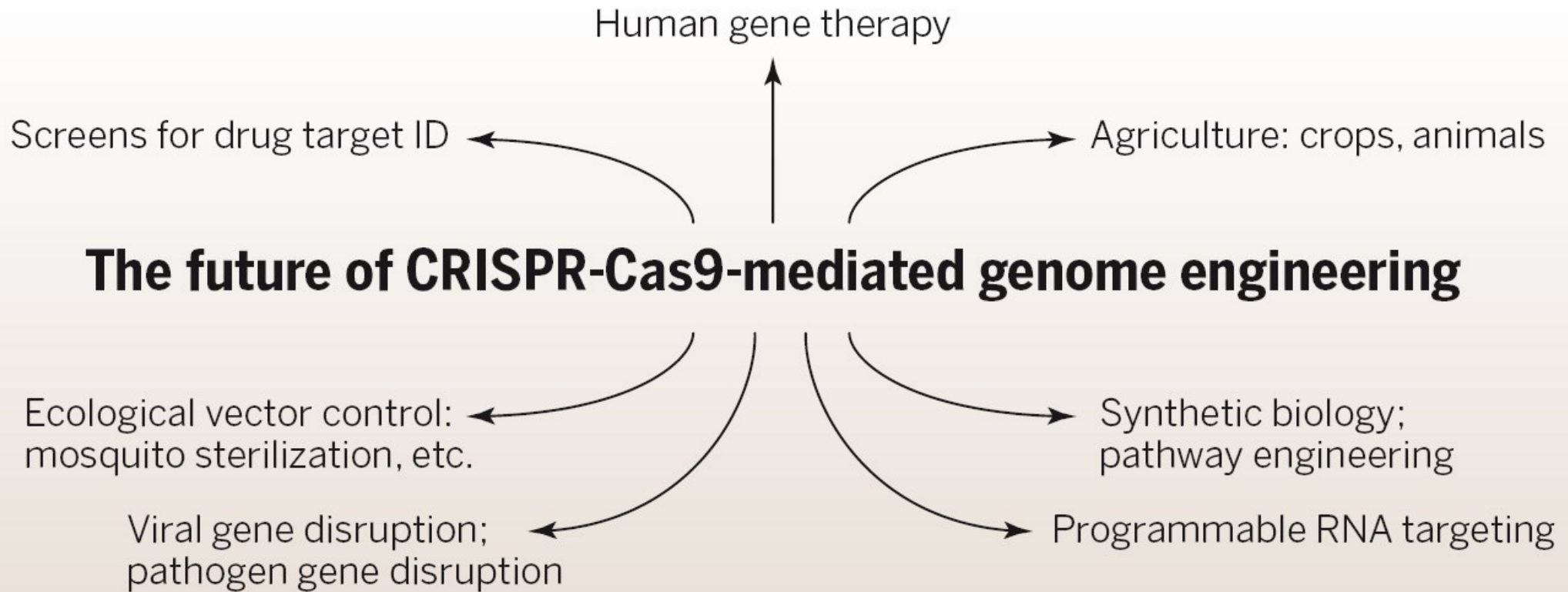
 **SAGE**
LABS
A Horizon Discovery Group Company

...vibrant development
and many many more

Things that changed everything



The future of CRISPR/Cas9



+ multiplexed genome editing targeting multiple sites

Thank you!