Winter School seminars

speaker	title	session	date	time	GT	TG e Neuro
Wenting Zhao NTU Singapore (online)	Engineered membrane deformation and guided reorganization of cellular machinery to study the mechanical properties of cells	Nuclear Integrity and chromatin organization	18/12/23	10-11.30	Colonnelli	Palermo
					Virgilio	Terzulli
					Majaliwa	Addario
						Roberto
						Iannella
						Valente
						Sileo
						Scansalegna
						Lollobrigida
					Jeong	Bartolucci
						Gigliotti
					Bernardi	Lupo
Fred Bernard Jacques Monod Institute, Paris, France	The importance of nucleus positioning in embryo development	Nuclear Integrity and chromatin organization	18/12/23	11.30-13	Colonnelli	Palermo
						Furina
						Sileo
						Lollobrigida
					Hazrati	Addario
					Bastianelli	
					Bernardi	Patriarca
Barbara Peruzzi IRCCS Children Hospital Bambino Gesù, Rome, Italy	Nuclear lamins and nuclear dysmorphism in pathologies through advanced microscopy lens	Nuclear Integrity and chromatin organization	18/12/23	14.00-15.30	Majaliwa	Scansalegna
						Sileo
					colonnelli	Moroni
					santacroce	Lollobrigida
						Iannella
						Roberto
						Piazza
						Caputo
					Fanelli	Gazzera
					Pace	Bertone
Jeremy Carlton King's College, London, UK	The ESCRT machinery at nuclear envelope: Closing holes and expanding roles	Nuclear Integrity and chromatin organization	18/12/23	15.30-17	Colonnelli	Radicioni
					Santacroce	Cutrona
					Pace	Pugliano
					Fanelli	Bertone
						Furina
						Gazzera
						Moroni
					Jeong	Dominici

Winter School seminars

Philippe Collas University of Oslo, Norway	D matters: epigenetic and chromatin conformation changes at multiple scales during adipocyte differentiation	Chromatin organization in development	19/12/23	10.00-11.30	Virgilio	Scaramagli
					Antonio	palermo
					Glaudo	Dominici
						de Rosa
						Patriarca
						Puleo
						Caputo
						Cutrona
						Piazza
Fulvio Chiacchiera, University of Trento	Role of chromatin-associated complexes during liver regeneration and tumor formation	Chromatin organization in development	19/12/23	11.30-13.00	Glaudo	Scaramagli
						Addario
						Scansalegna
						Meoni
						Patriarca
						Puleo
						De Rosa
						Furina
						Piazza
						Chillura
Daniela Palacios Cattolica University of Rome	Epitranscriptomics in muscle development and disease	Chromatin organization in development	19/12/23	14.00-15.30	Pace	Bertone
					Bartolini	Meoni
					Majaliwa	Scansalegna
						Patriarca
						Puleo
						Furina
					Pourali	Pugliano
						Valente
Matthieu Boulard European Molecular Biology Laboratory EMBL, Rome	Sweet gene regulation in the mammalian embryo	Chromatin organization in development	19/12/23	15.30-17.00		Cutrona
					Antonio	Bertone
					Pourali	Radicioni
					Bastianelli	Chillura
					Hazrati	Valente
					Bartolini	Scansalegna
					Pace	

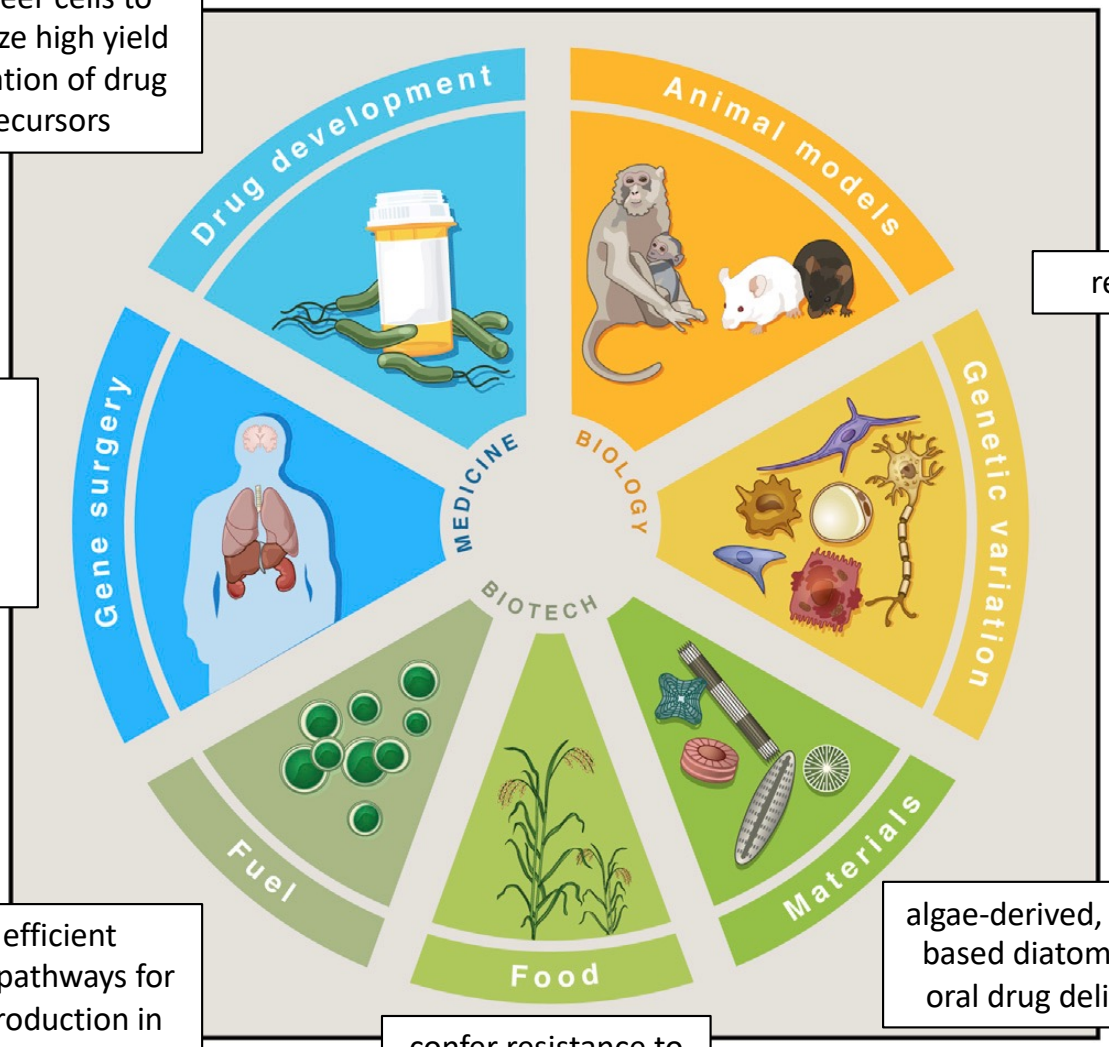
Winter School seminars

Jamie Hackett European Molecular Biology Laboratory EMBL, Rome	What do chromatin modifications do? Epigenome editing to dissect function in health and disease?	Genome Stability in mouse model of diseases	20/12/23	10.00-11.30	Hazrati	Palermo
					Antonio	Scaramagli
					Pourali	Meoni
						Sileo
						Cutrona
						Iannella
						Roberto
						Dominici
						Lollobrigida
						terzulli
						Bartolucci
					Ilie	Gigliotti
Monica Ballarino Sapienza University of Rome	Myogenesis and long non-coding RNAs: a chromatin affair	Genome Stability in mouse model of diseases	20/12/23	11.30-13.00	Fanelli	Scaramagli
					Virgilio	Gigliotti
					Glaudo	Piazza
						de rosa
						iannella
						Puleo
						Roberto
					Ilie	bartolucci
						Terzulli
Marina Vietri University of Oslo, Norway	Nuclear envelope dynamics at ruptured micronuclei	Genome Stability in mouse model of diseases	20/12/23	14.00-15.30	Jeong	Meoni
					Bernardi	gigliottti
					Fanelli	Dominici
					Majaliwa	Chillura
					santacroce	Lupo
					Glaudo	Radicioni
						Pugliano
						Caputo
					Hazrati	Moroni
					Bartolini	Gazzera
Rafal Czapiewski University of Edinburgh, UK (online)	Nuclear envelope controls genetic spacetime - focus on genome organization and function.	Genome Stability in mouse model of diseases	20/12/23	15.30-17.00		Radicioni
					Antonio	Pugliano
					Pourali	Caputo
					santacroce	addario
						de rosa
						Cutrona
					Bartolini	Moroni
					Bernardi	Gazzera

Genome editing, called also genome engineering,
processes of making targeted modifications to the genome,
its contexts (e.g., epigenetic marks), or its outputs (e.g., transcripts).

Genome engineering technologies are enabling a broad range of applications

engineer cells to optimize high yield generation of drug precursors



reverse genetic

direct *in vivo* correction of genetic or epigenetic defects in somatic tissue

create efficient metabolic pathways for ethanol production in algae or corn

confer resistance to environmental deprivation or pathogenic infection

algae-derived, silica-based diatoms for oral drug delivery

(Hsu et al., Cell, 2014)

Classic Definition of Gene Therapy:

transfer of genetic material to a patient to treat a disease

AIM:

2.0 Gene Therapy

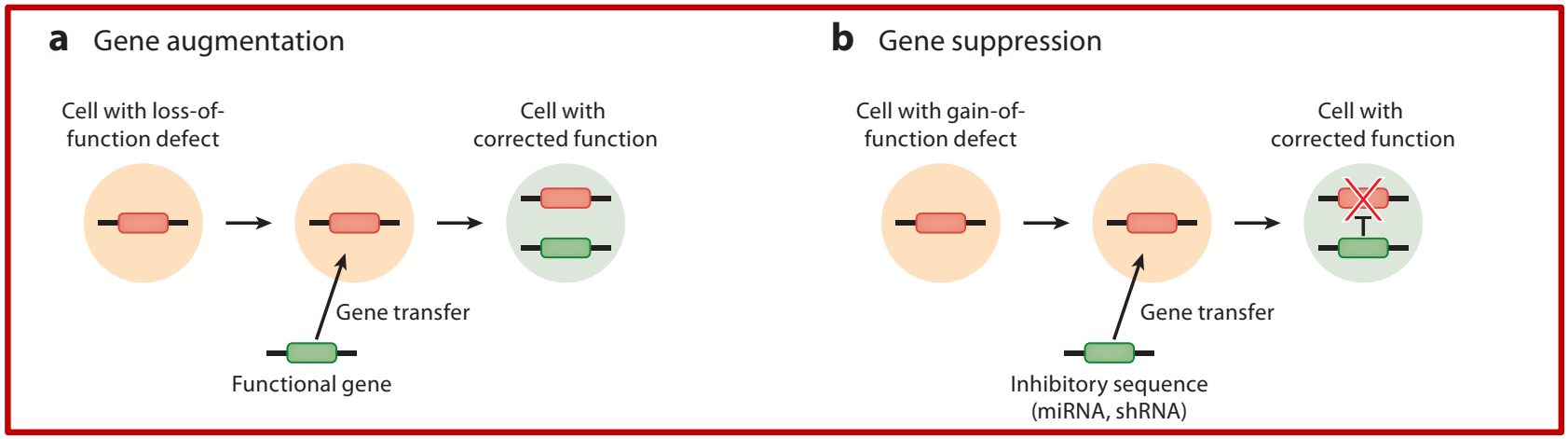
long- term expression of the transferred gene high enough to be therapeutic

3.0 Gene Therapy (gene editing)

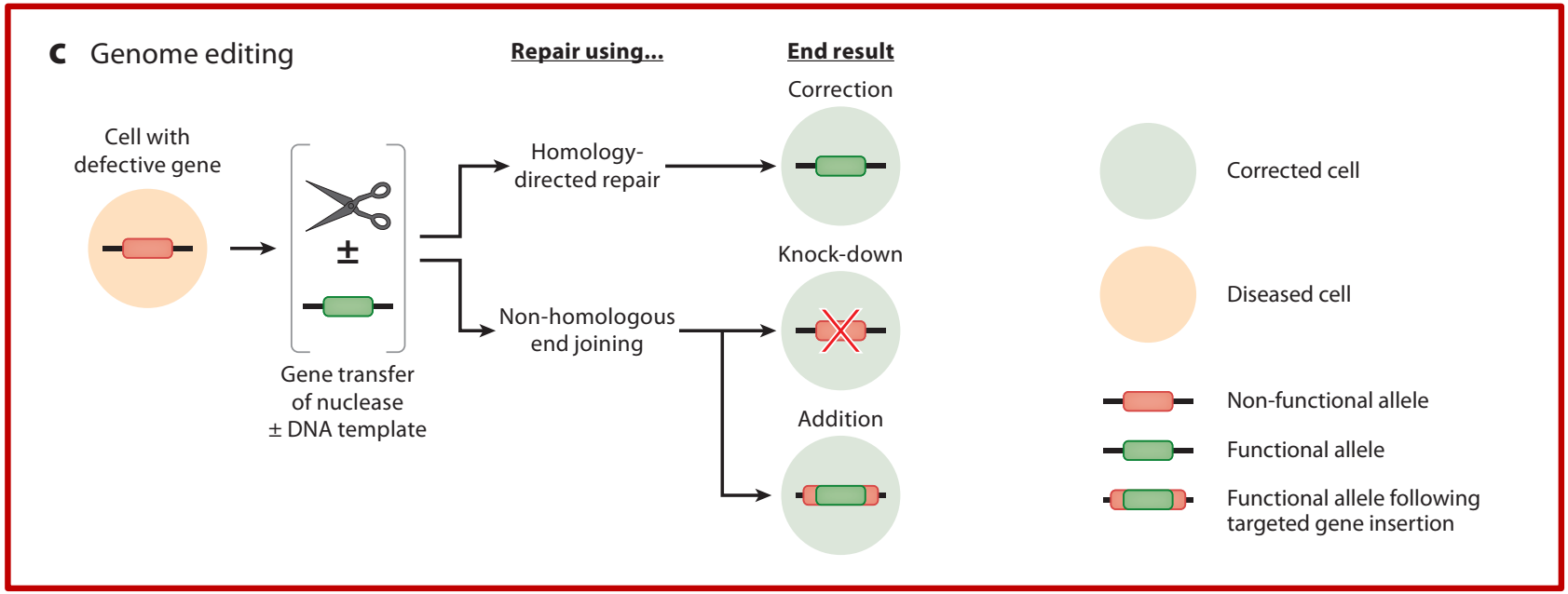
long- term correction of the 'edited' gene high enough to be therapeutic

2.0 gene therapy vs 3.0 gene therapy

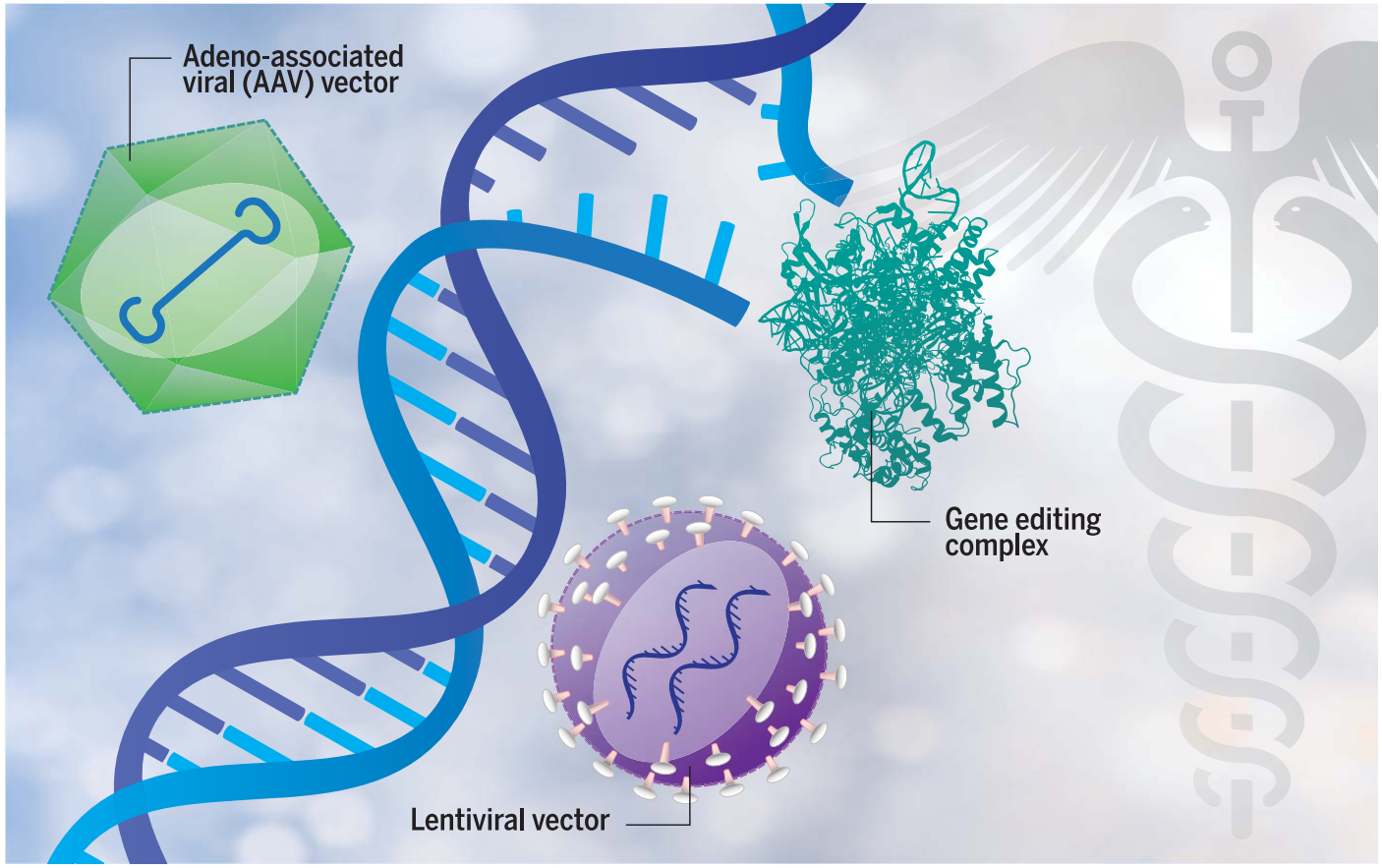
2.0



3.0



(Xavier M. Anguela and Katherine A. High, Annual Reviews of Medicine 2018)



(Fazhan Wang et al., J Gene Med. 2019)

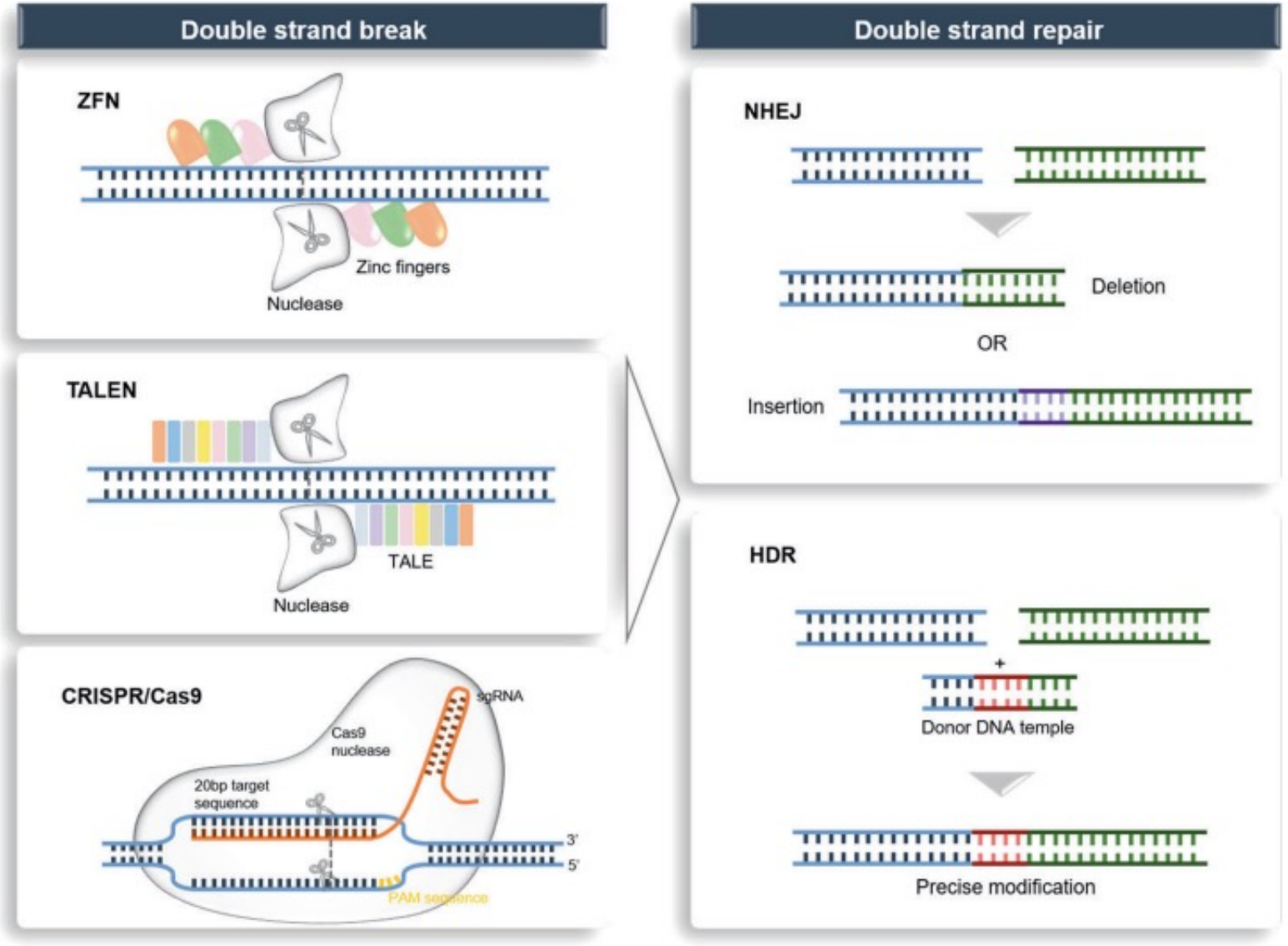
Genome editing tools:

Used since:

2001

2010

2013



Li et al, Signal Transduction and Targeted Therapy (2020)

Genome editing tools:

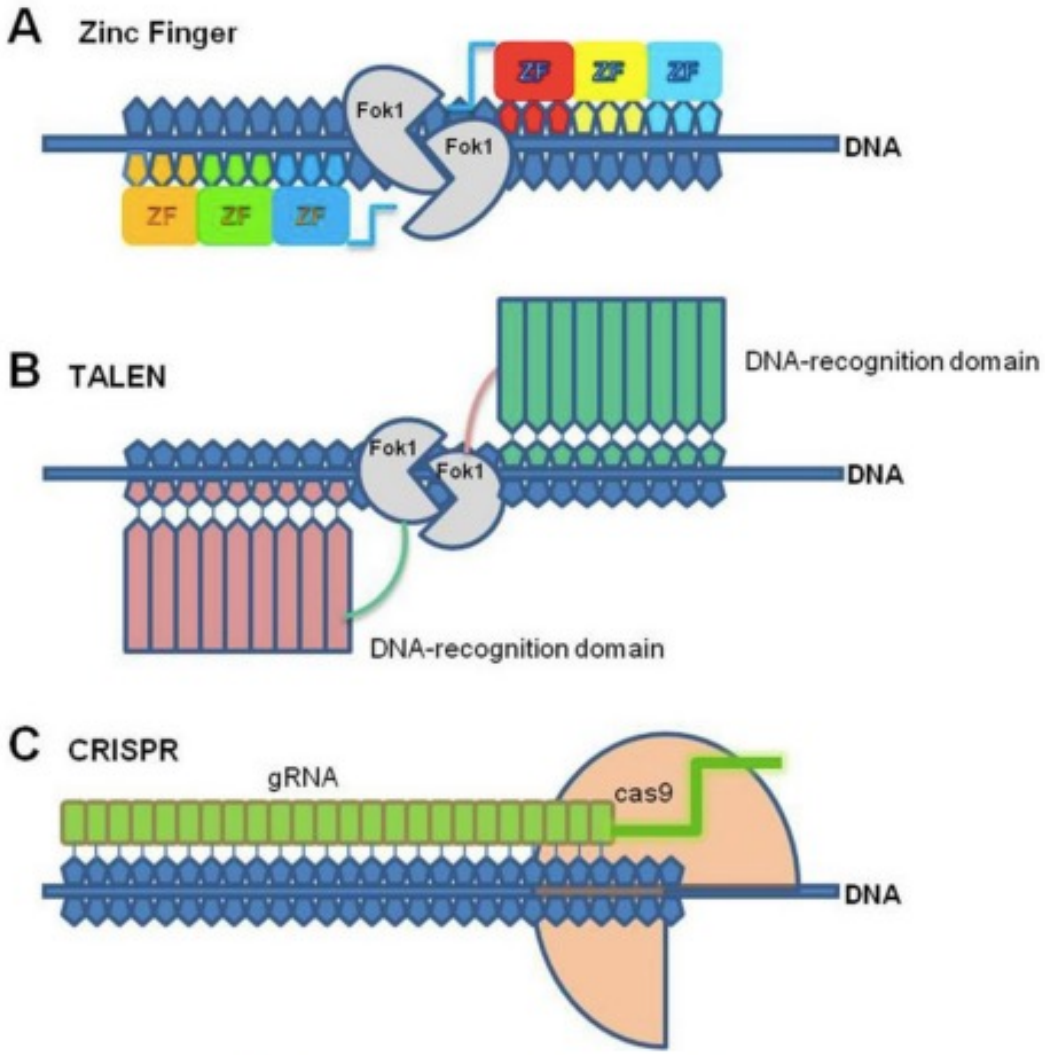
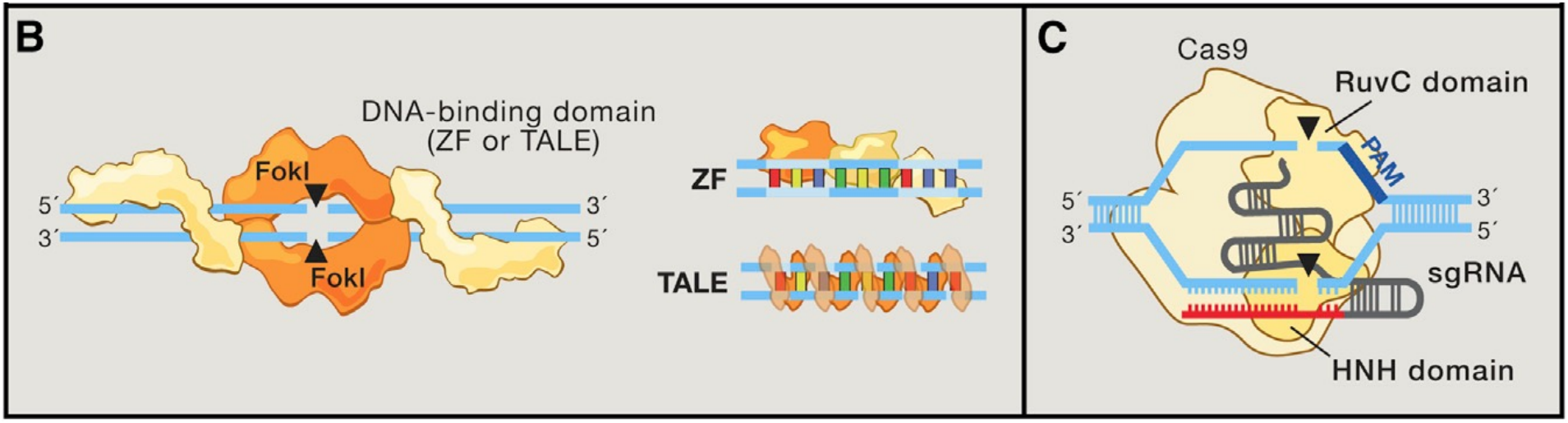


Fig. 1. Schematic representation of Genome Editing Tools.

Sanagala et al, Journal of Genetic Engineering and Biotechnology (2017)

Genome editing tools:

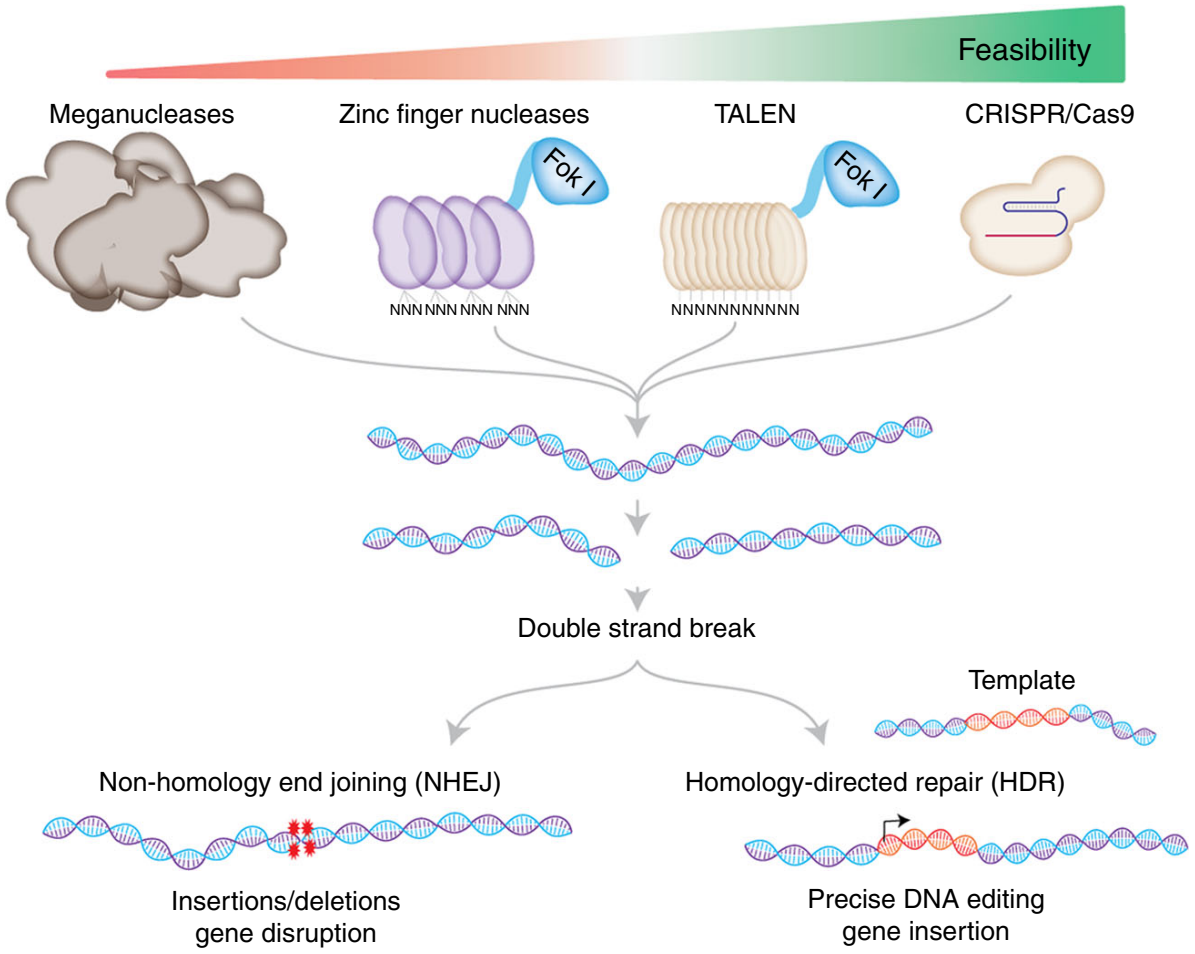


(Hsu et al., Cell, 2014)

	TALEN and ZFN	CRISPR/Cas9
Target binding principle	Protein-DNA specific recognition	Watson-Crick complementary rule
Working mode	Specifically recognizes the target DNA and dimeric Fok1 makes DSB	Guide RNA specifically recognizes the target DNA and Cas9 makes DSB
Essential components	Dimers of TALE/ZFN-Fok1 fusion protein	Guide RNA and Cas9
Target DNA lenght	14-18 bp	20 bp
Time consumption for construction	5-7 days	1-3 days
Multiple targeting	context-dependent binding (multiple proteins)	high specificity with multiple sgRNAs

(Adapted from Wei C. et al., Journal of Genetics and Genomics, 2013)

CRISPR/Cas9 technology increased the feasibility of genome-editing technologies



(Adli M., Nature communications, 2018)

CRISPR/Cas9

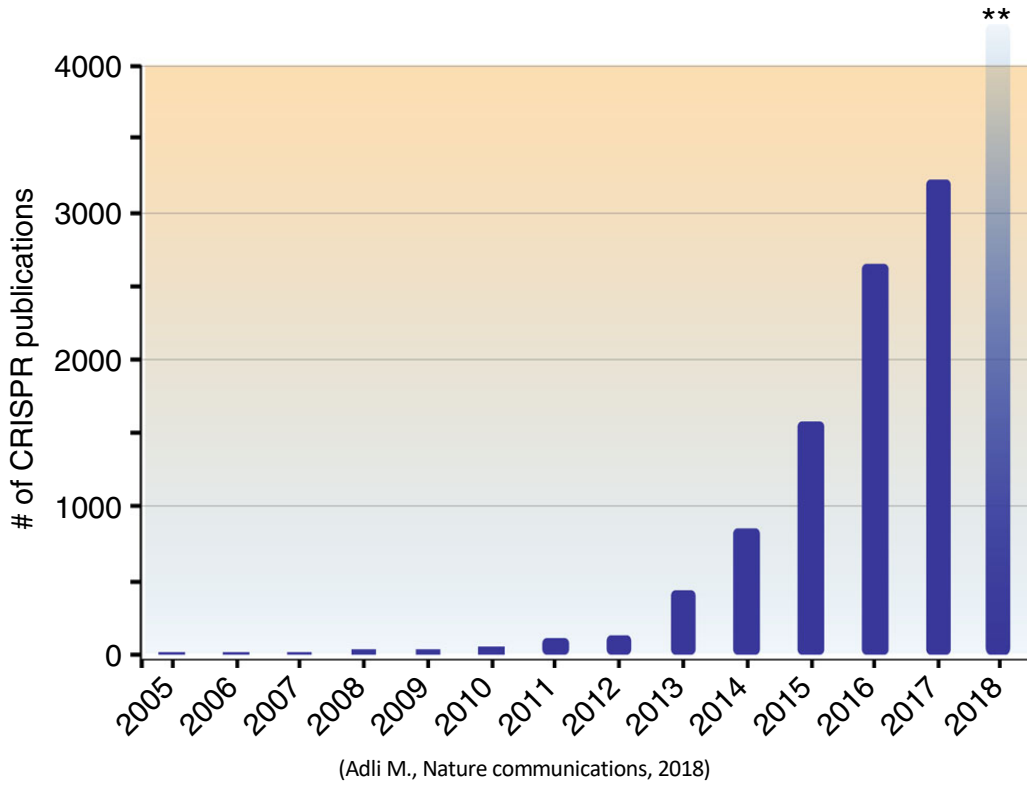
CRISPR/Cas9 - It all started with yogurt

2005-Rodolphe Barrangou discovered that S. thermophilus contained odd chunks of repeating DNA sequences—Crisprs

CRISPR/Cas9 - as a tool for genetic engineering

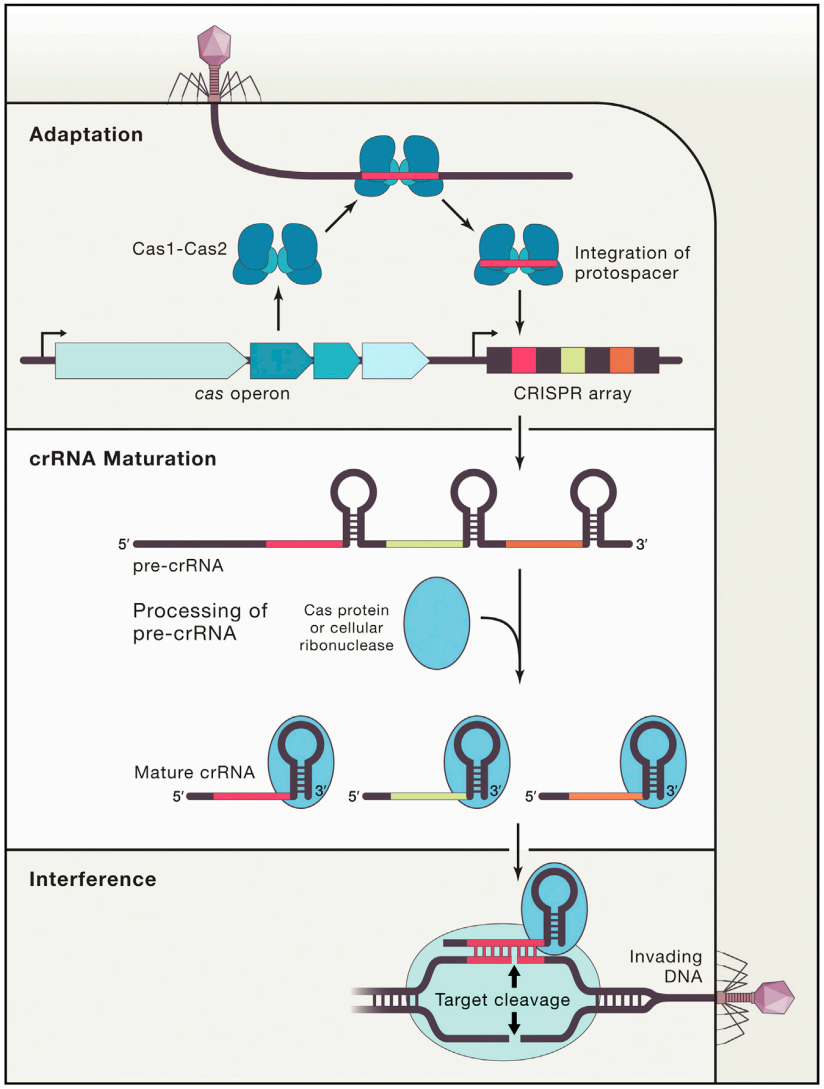
2012 : Jennifer Doudna and Emmanuelle Charpentier discovered S. pyogenes molecular mechanism

CRISPR/Cas9 technology increased the feasibility of genome-editing technologies



(Adli M., Nature communications, 2018)

CRISPR system in prokariotes is an adaptive immunity system



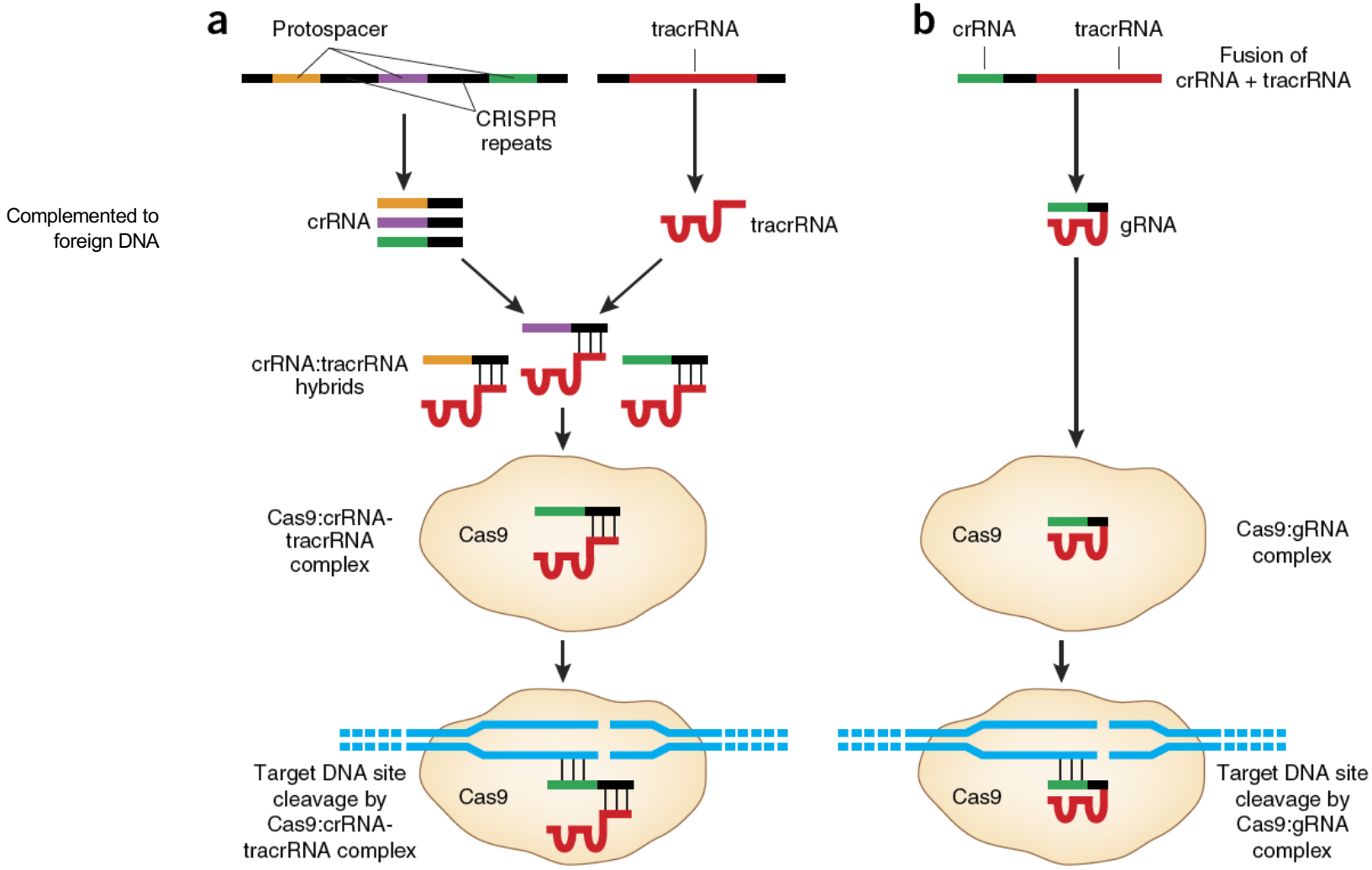
(Hille F. et al., Cell, 2018)

Engineered CRISPR-Cas9 system consists of a fusion between a crRNA and a part of the tracrRNA sequence: sgRNA

CRISPR system in prokariotes is an adaptive immunity system

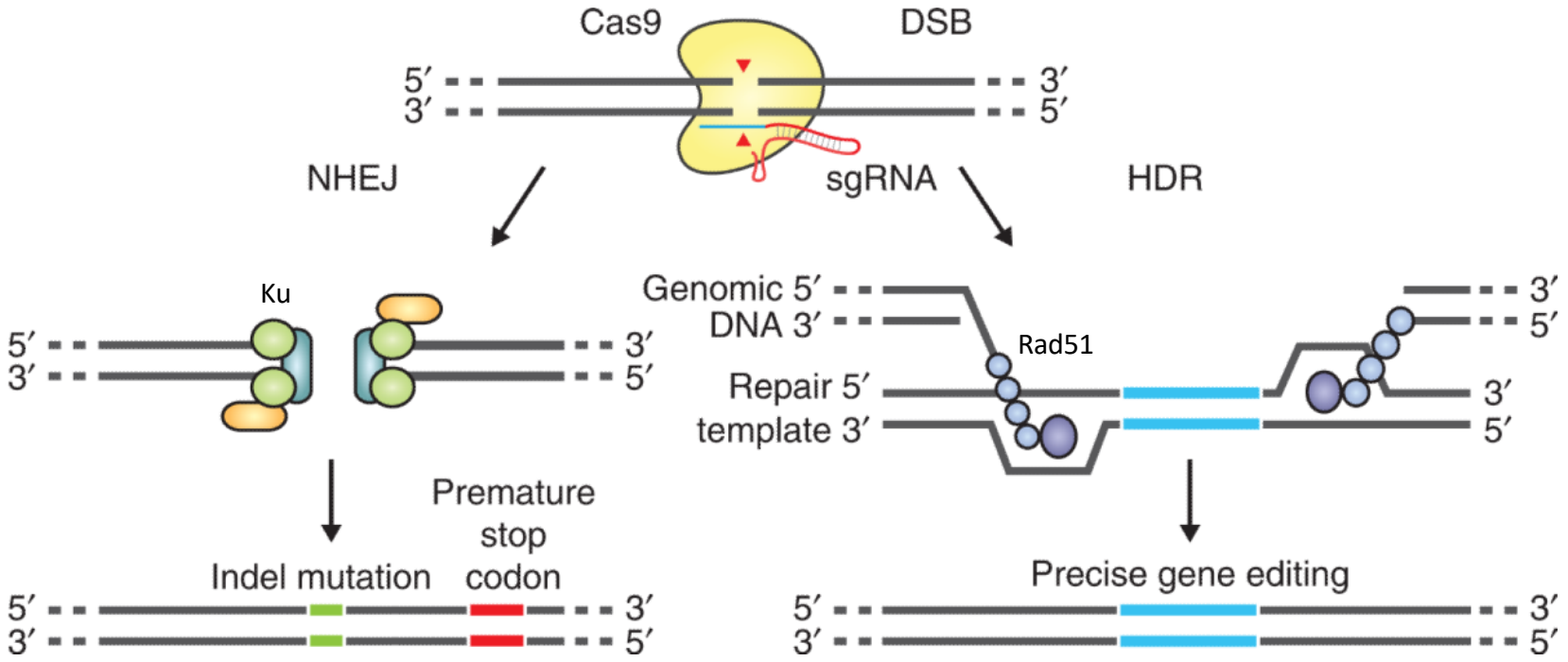
Naturally occurring CRISPR-Cas9 systems

Engineered CRISPR-Cas9 systems



(Sander D. and Joung K., nature biotechnology, 2014)

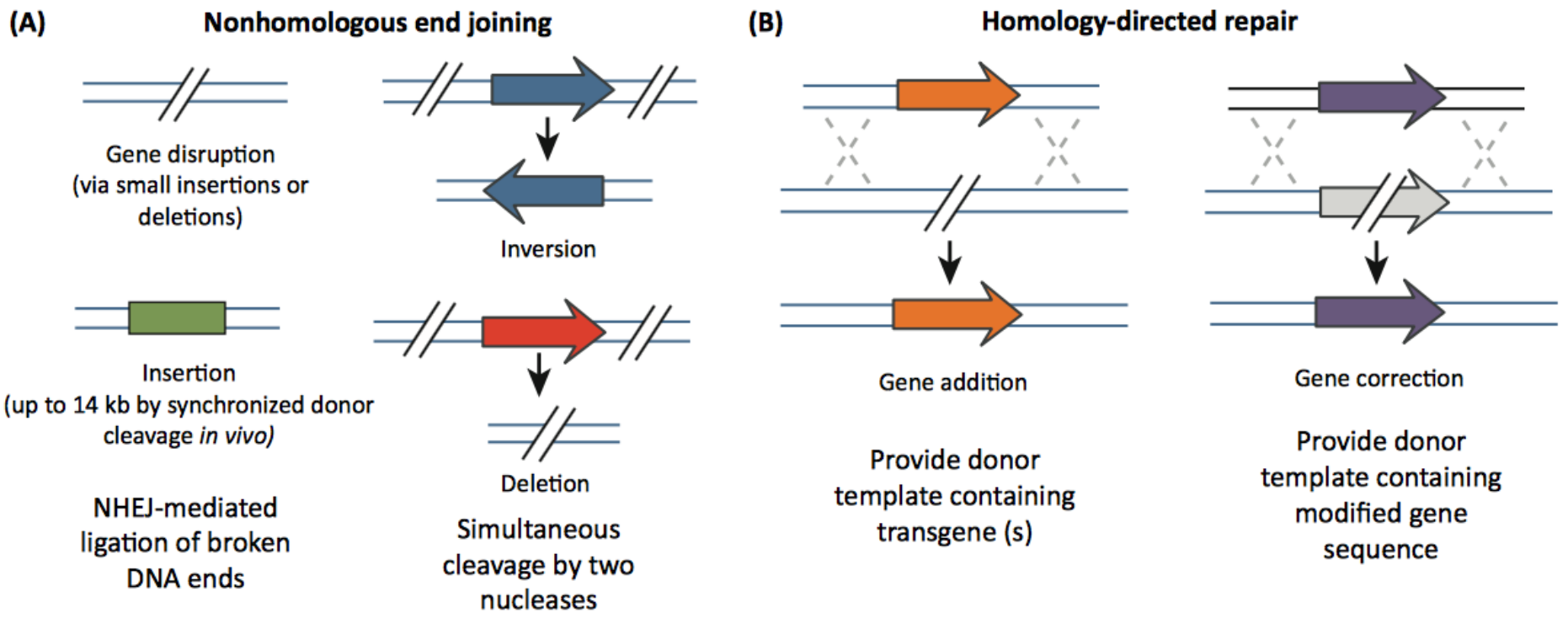
CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery



(Ran et al, Nat Protoc. 2013)

Insertion/deletion mutations=indels

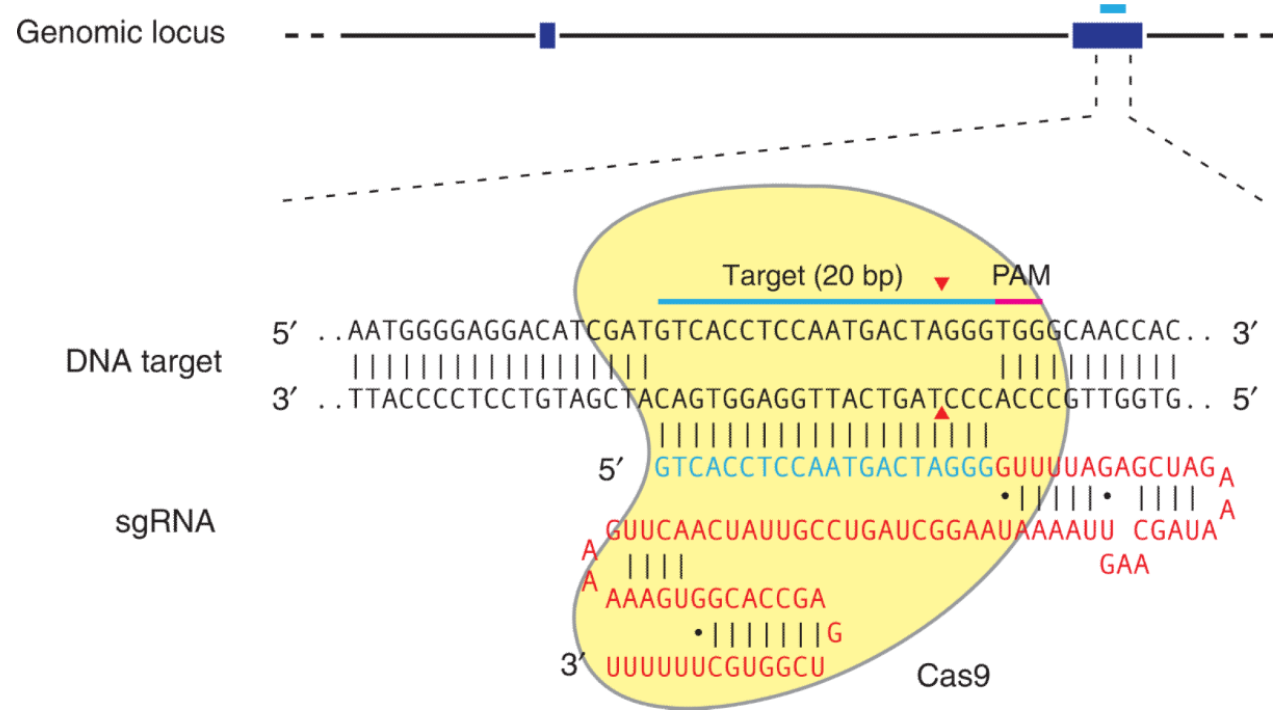
CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery



(Gaj T. at al., Trends Biotechnol, 2013)

Cas9 nuclease from *S. pyogenes* is targeted to genome by an sgRNA consisting of a 20-nt guide sequence and a scaffold

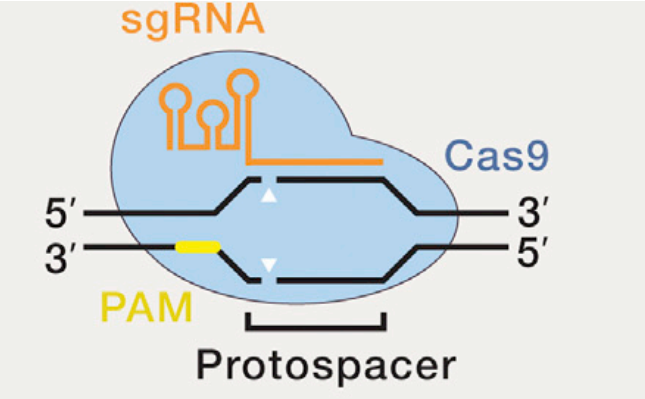
Genetic GPS



(Ran et al, Nat Protoc. 2013)

The only restriction for targeting is that the sequence must be followed by **PAM motif**

RNA-programmed endonucleases offer a variety of genome editing-options



SpCas9:

- More characterized;
- Balance between PAM complexity and construct size;
- Tested in a variety of contexts

(Komor A.C. et al., Cell, 2017)

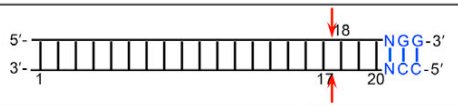
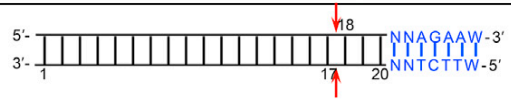
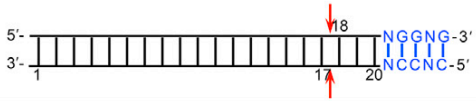
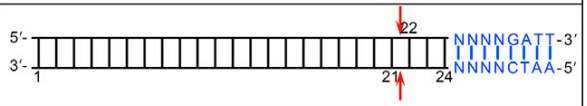
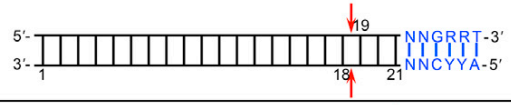
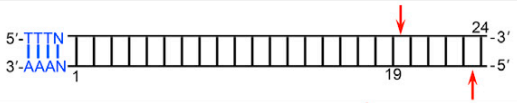
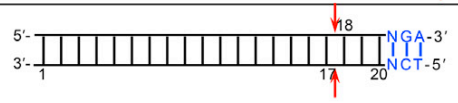
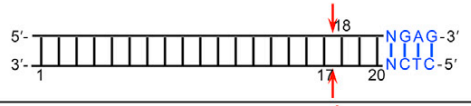
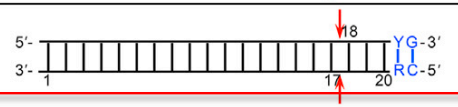
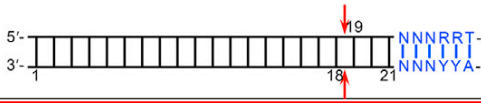
Enzyme name	Size (residues)	PAM requirement and cleavage pattern
SpCas9 / FnCas9	1368 / 1629	5′-N(1)…N(17)N(18)NGG-3′ / 3′-NCC-5′ (20)
St1Cas9	1121	5′-N(1)…N(17)N(18)NNAGAAW-3′ / 3′-NNTCTTW-5′ (20)
St3Cas9	1409	5′-N(1)…N(17)N(18)NGGNG-3′ / 3′-NCCNC-5′ (20)
NmCas9	1082	5′-N(1)…N(21)N(22)NNNNGATT-3′ / 3′-NNNNCTAA-5′ (24)
SaCas9	1053	5′-N(1)…N(18)N(19)NNGRRT-3′ / 3′-NNCYYA-5′ (21)
AsCpf1 / LbCpf1	1307 / 1228	5′-TTTN-3′ / 3′-AAAN-5′ (1); N(19)…N(24)
VQR SpCas9	1368	5′-N(1)…N(17)N(18)NGA-3′ / 3′-NCT-5′ (20)
EQR SpCas9	1368	5′-N(1)…N(17)N(18)NGAG-3′ / 3′-NCTC-5′ (20)
VRER SpCas9	1368	5′-N(1)…N(17)N(18)NGCG-3′ / 3′-NCGC-5′ (20)
RHA FnCas9	1629	5′-N(1)…N(17)N(18)YG-3′ / 3′-RC-5′ (20)
KKH SaCas9	1053	5′-N(1)…N(18)N(19)NNNRRT-3′ / 3′-NNNYYA-5′ (21)

RNA-programmed endonucleases offer a variety of genome editing-options

PRO	CONS
- Target design simplicity; - Higly efficiency - Fast (4 weeks for mice);	- **fidelity (off-targets, effects)** - **delivery** - **targeting scope**

- OPEN QUESTIONS:
- **Immunogenicity of nucleases *in vivo* (?)**
- **Ethics (?)**

I - targeting scope

Enzyme name	Size (residues)	PAM requirement and cleavage pattern
SpCas9 / FnCas9	1368 / 1629	
St1Cas9	1121	
St3Cas9	1409	
NmCas9	1082	
SaCas9	1053	
AsCpf1 / LbCpf1	1307 / 1228	
VQR SpCas9	1368	
EQR SpCas9	1368	
VRER SpCas9	1368	
RHA FnCas9	1629	
KKH SaCas9	1053	

RHA FnCas9 requires only a YG PAM

(Komor A.C. et al., Cell, 2017)

KKH SaCas9 shows Relaxed PAM specifities

II - fidelity

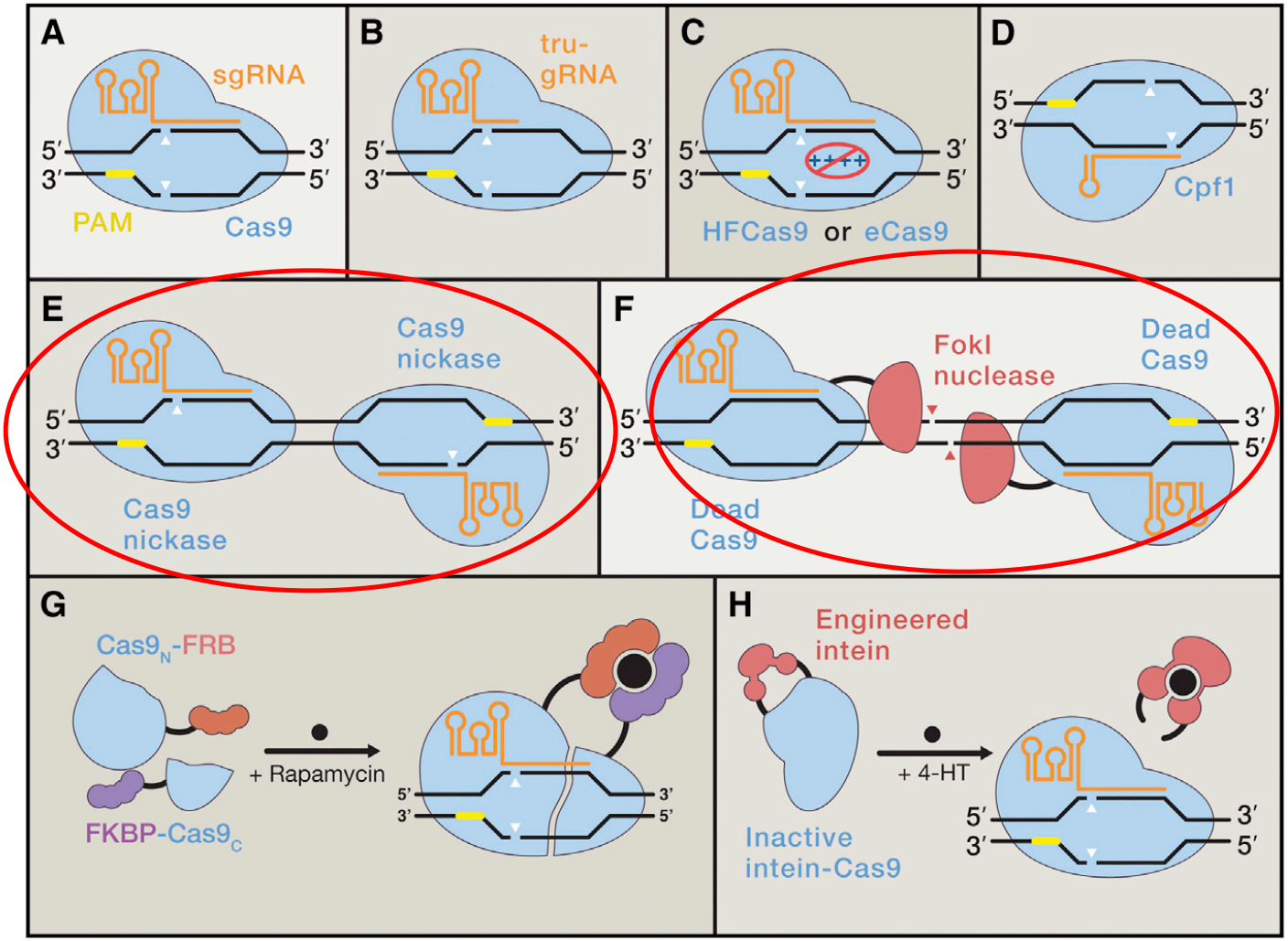
How to check?

- Whole genome deep sequencing;
- BLESS
- GUIDE-Seq
- Digenome-Seq

II - Fidelity

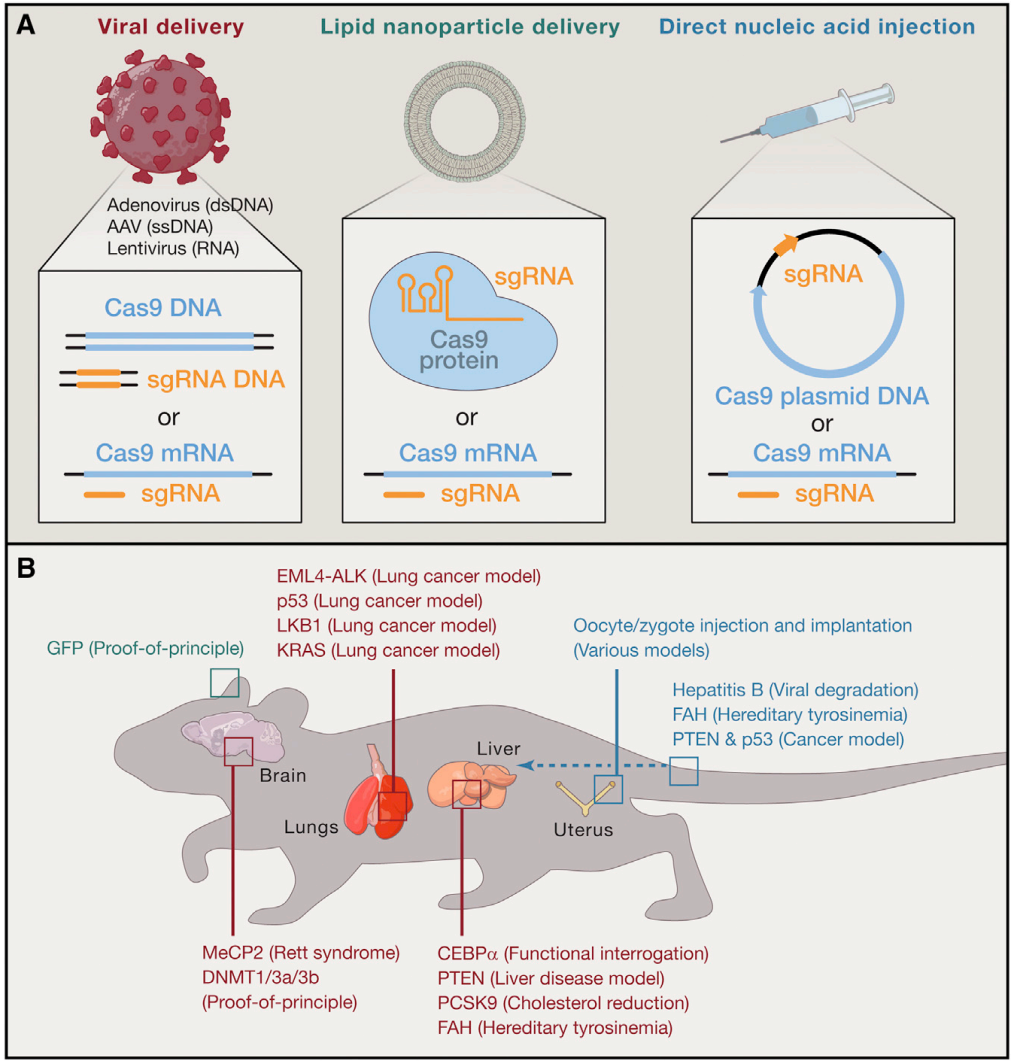
How to improve?

Reduction of off-targets



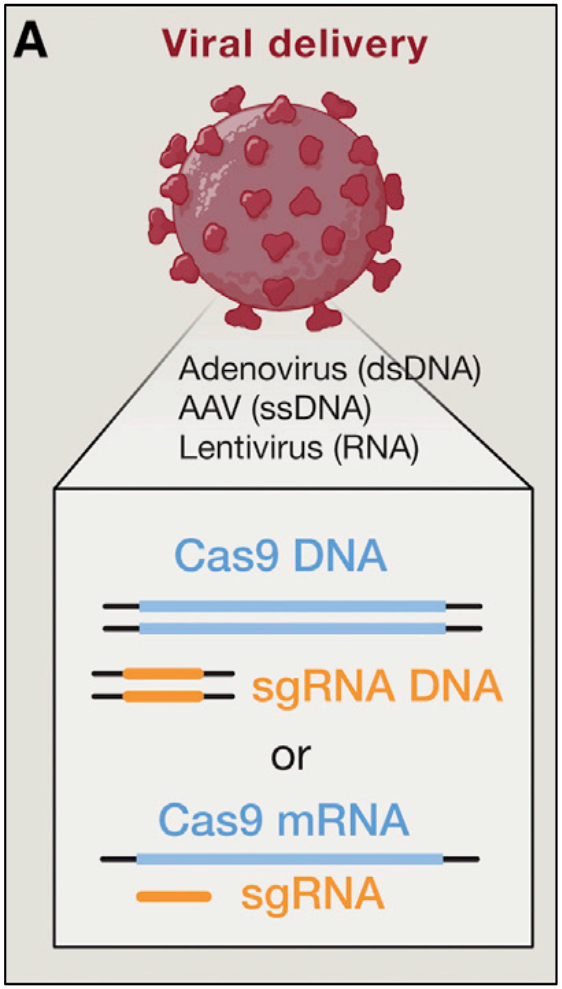
(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

Lentivirus:

- infects non dividing cells;
- Packaging limit **~8.5 kb** (package Cas9 genes, gRNA, promoter and regulatory sequences)

Adenovirus:

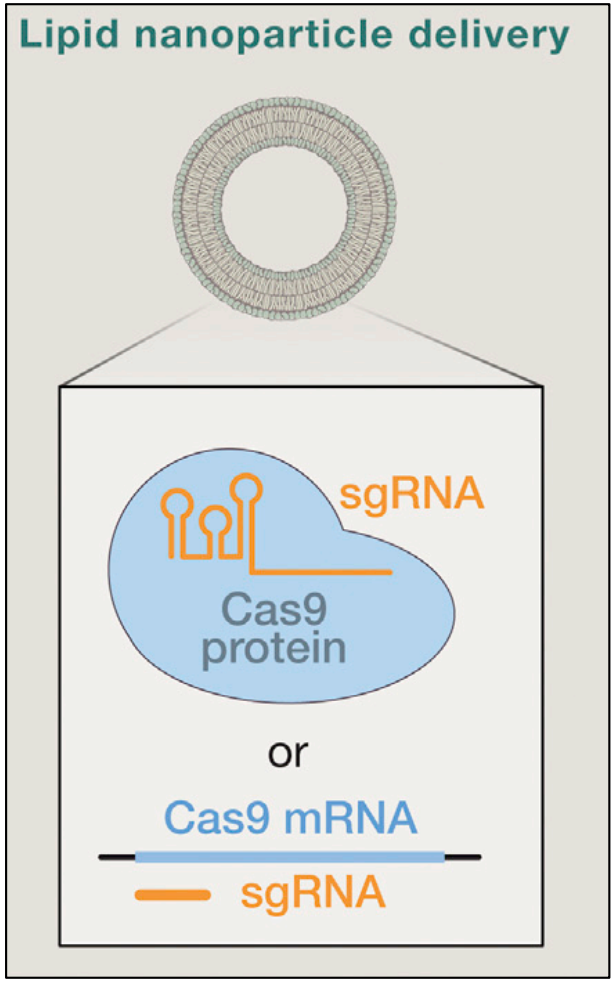
- infects dividing and non dividing cells;
- Do not integrate DNA;
- Elicits strong immune response in animals;

AAV variants:

- infect both dividing and non-dividing cells;
- do not integrate;
- do not elicit immune response in the host;
- A variety of serotypes of AAV are known,

- AAV has a packaging limit of **~4.5** kb of foreign DNA

III – delivery

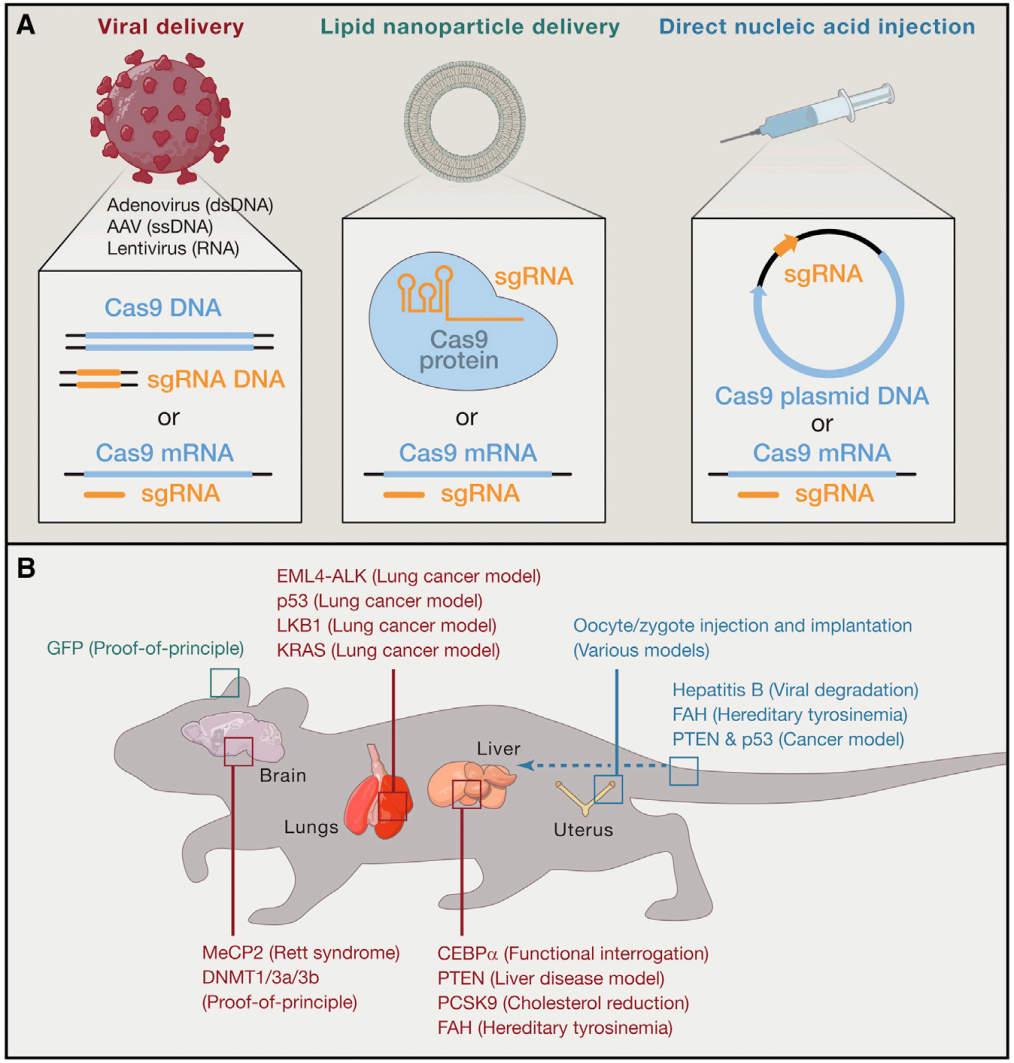


Lipid nanoparticle delivery:

- more transient
- higher DNA specificity
- less off-target editing

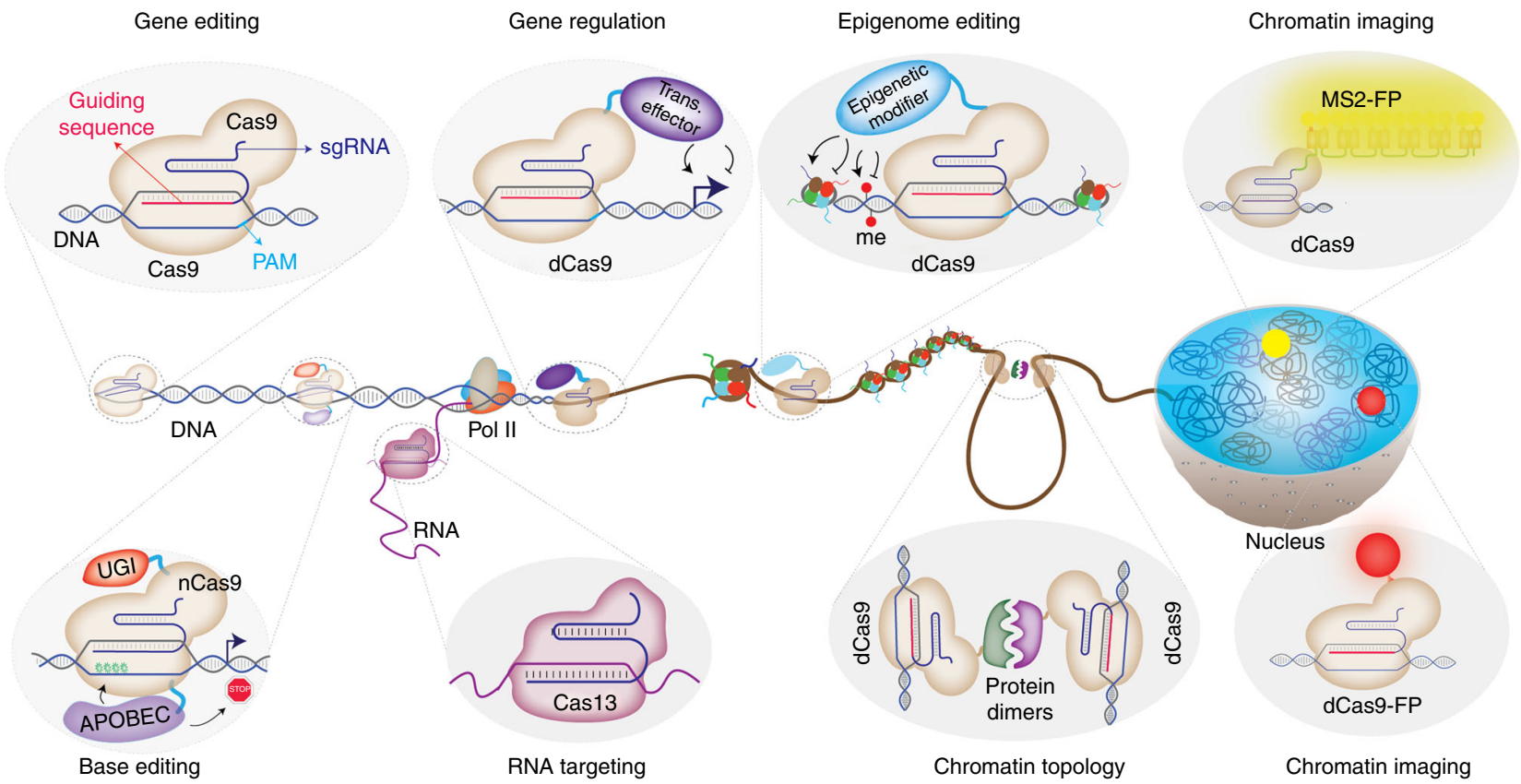
(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

CRISPR/Cas9 technologies beyond genome editing are based mainly on dead-Cas9



(Adli M., Nature communications, 2018)