力鈞生物科技有限公司
ZGene biotech Inc.

費司科技有限公司
Faith Technology Co., Ltd.

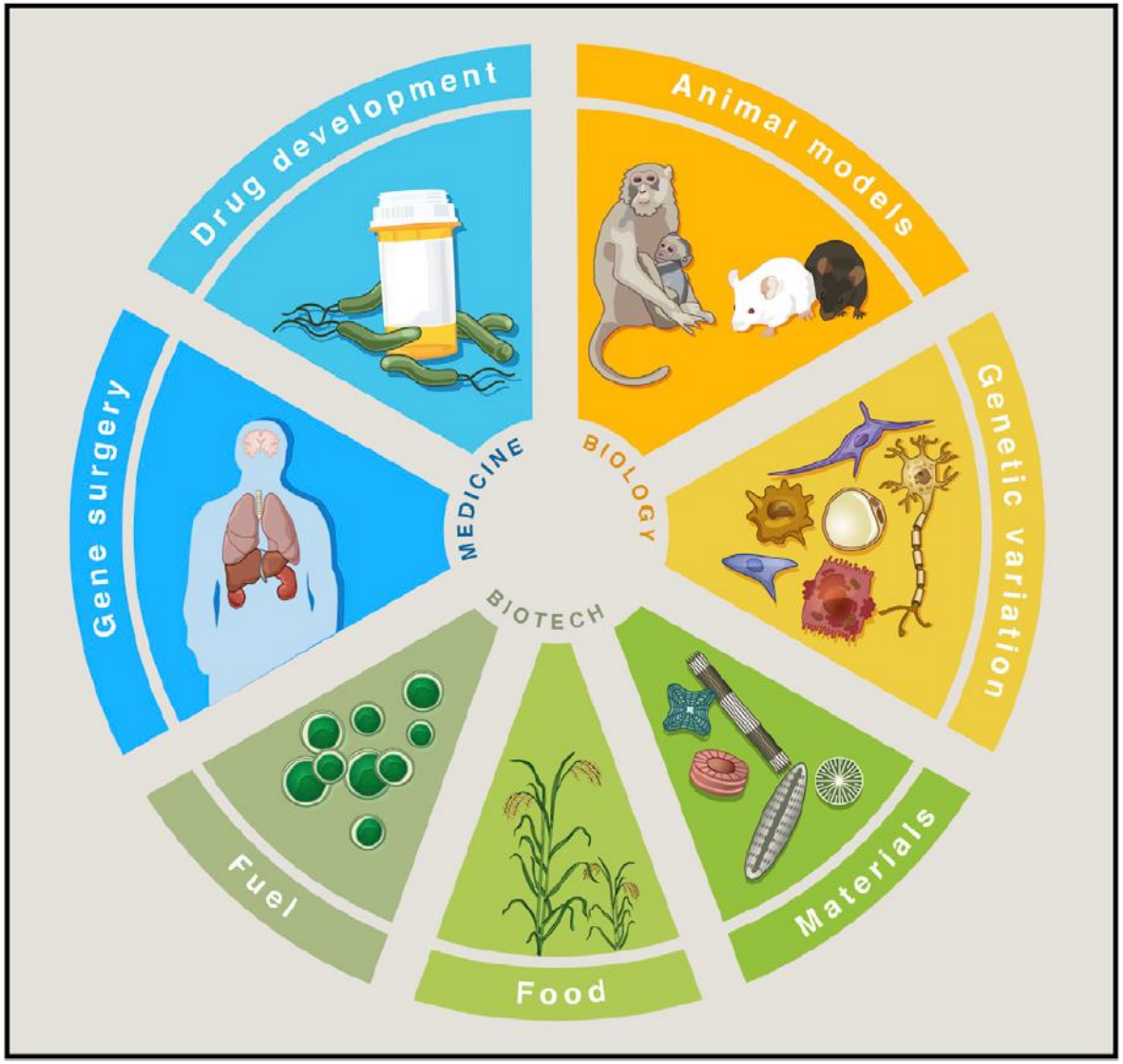
Genome Editing Tools
基因編譯技術介紹

力鈞生物科技 技術總監
許耿豪 博士

Genome Editing

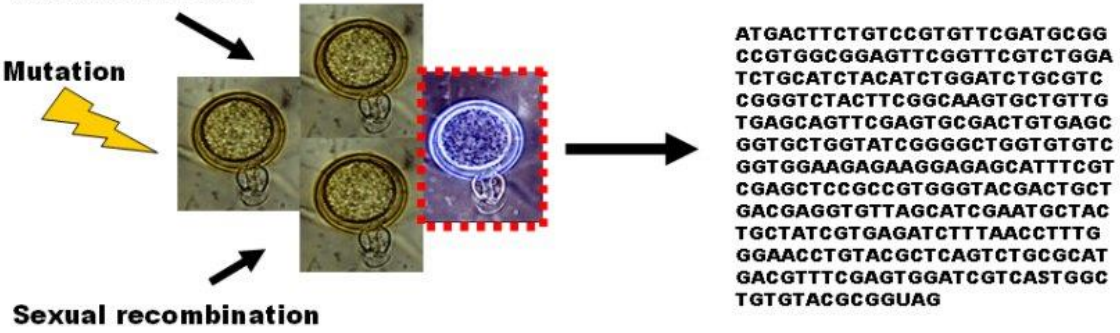
Development and Application

Applications of Genome Engineering

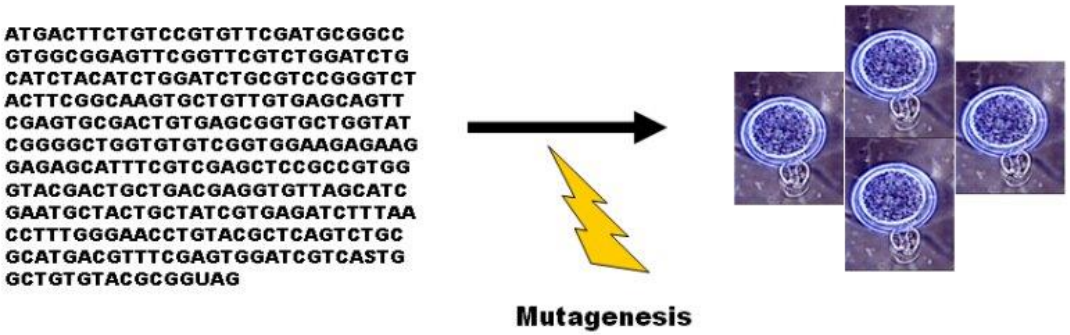


Functional genomic analysis

Forward Genetics



Reverse Genetics



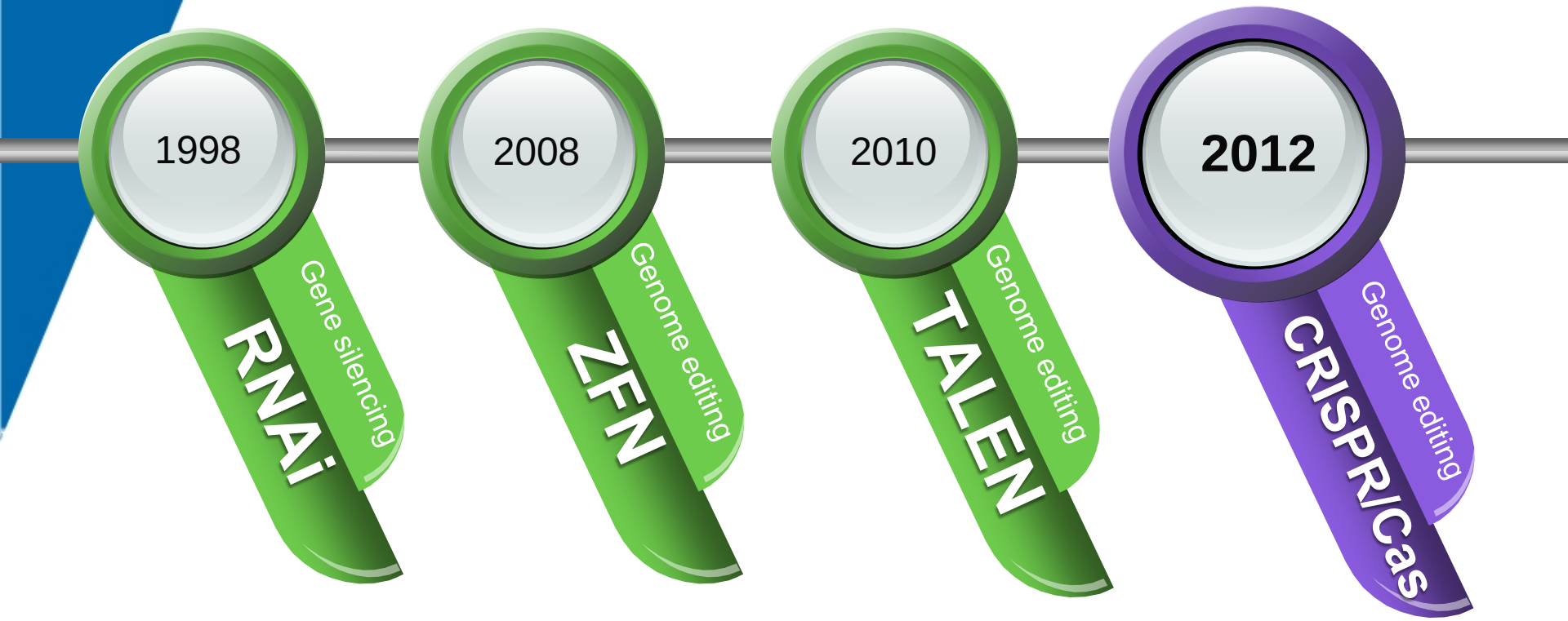
Reverse genetics in eukaryotes

Table 1 | Strategies to manipulate genes or gene expression in eukaryotes

–, not applicable; +/–, proof-of-principle; +, small-scale collections; ++, service for the research community; +++, genome-wide collections.

	Organism						
Strategy	**Yeast**	***A. thaliana***	***C. elegans***	**Fruitfly**	**Zebrafish**	***Xenopus***	**Mouse**
Insertion mutagenesis	–	+++ Genome-wide collection of T-DNA mutants	+++ Genome-wide collection of *Mos1* mutants in progress	+++ Genome-wide collection of P element and piggyBac mutants	+ Proofs-of-principle for retrovirus and transposon-mediated mutagenesis	+/–	+++ Genome-wide collection of gene-trap ES cells in progress
Random chemical mutation and screening	– (used in forward, not reverse genetics)	+++ Genome-wide collection of TILLING mutants, service	+ Middle-scale collection of TILLING mutants	++ Service for the research community	++ Service for the research community	+/– Proof-of-principle	–
Homologous recombination	+++ Genome-wide collection of KO and KI mutants	–	+/– Few examples	+ Several examples, but no systematic approach	–	–	+++ Genome-wide collection of cKO ES cells in progress
Inserted element-mediated gene engineering	–	–	++ MosTIC, MosSCI, MosDEL	+ Transposon-controlled deletions	–	–	–
RNAi	–	++	+++ Genome-wide 'feeding library'	+++ Genome-wide UAS-driven library	–	–	+/– Few examples
MO	–	–	–	–	++ Middle-scale screening	++ Middle-scale screening	

Specific Reverse Genetic approaches



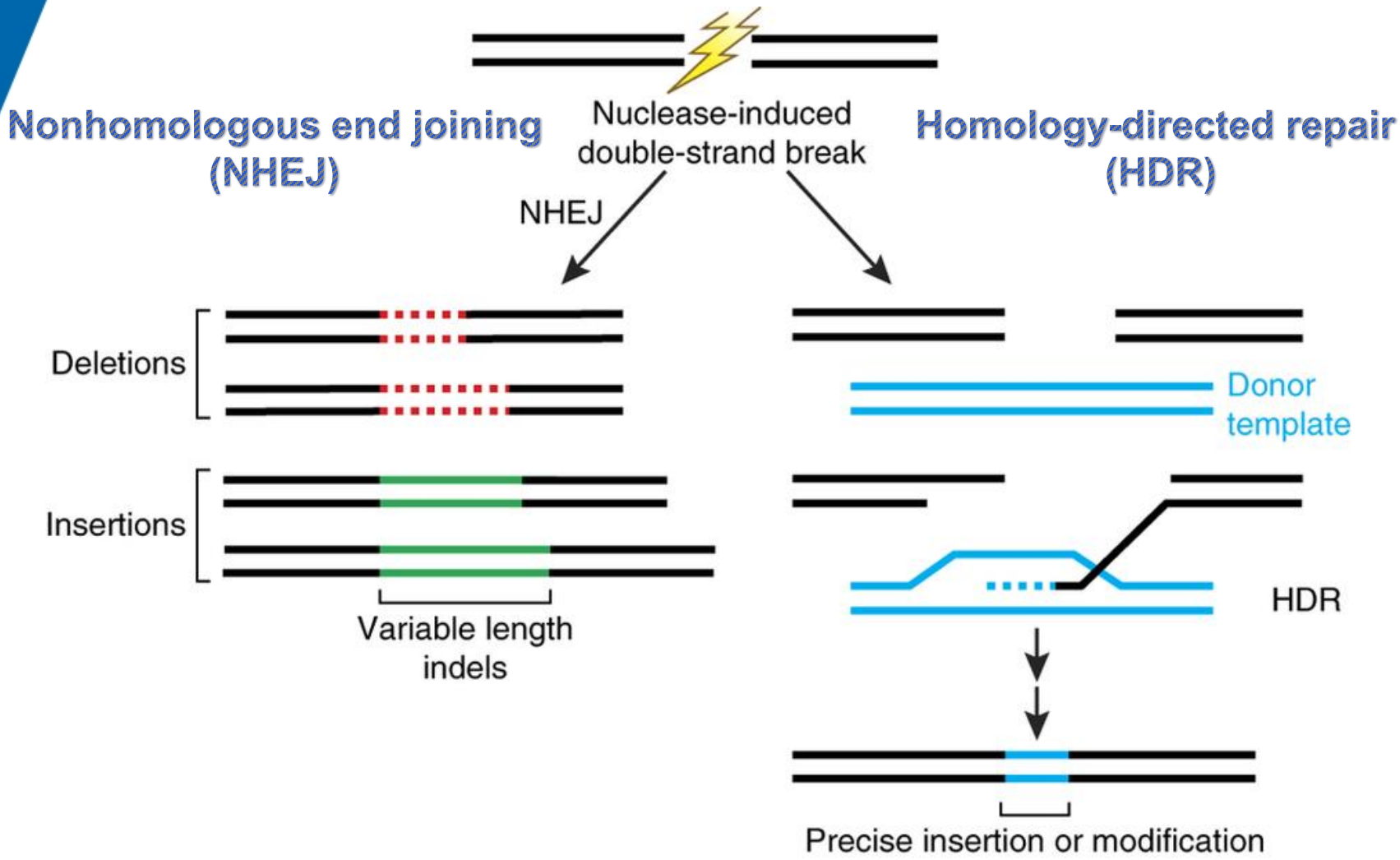
Classical and contemporary approaches for establishing gene functions

Genome Editing

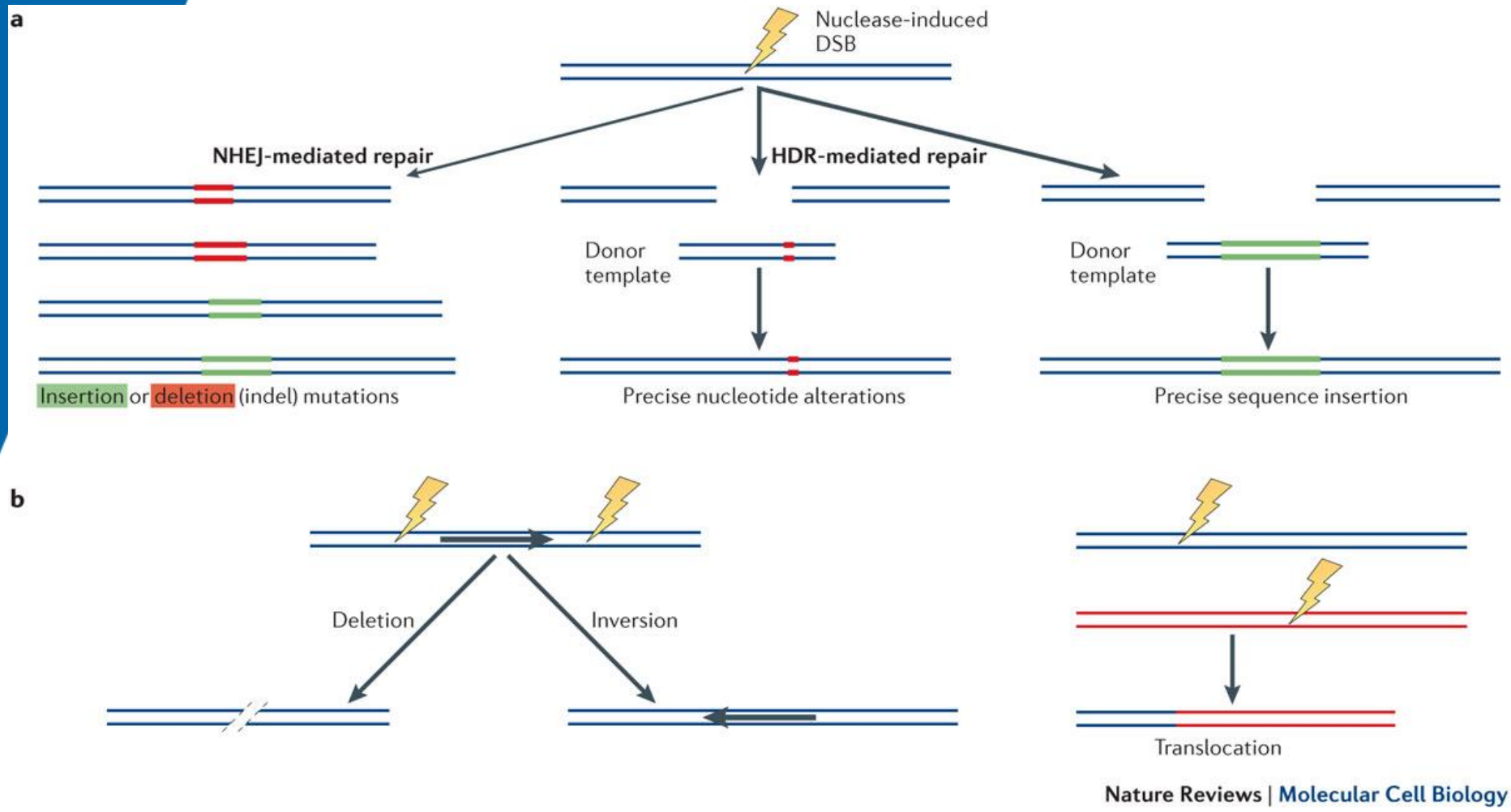
Genome editing is a type of genetic engineering in which DNA is **inserted**, **replaced**, or **removed** from a genome using artificially engineered nucleases, or "molecular scissors".

http://en.hdbuzz.net

NHEJ and HDR


Nonhomologous end joining
(NHEJ)
Nuclease-induced
double-strand break
Homology-directed repair
(HDR)
NHEJ
Deletions
Insertions
Variable length
indels
Donor
template
HDR
Precise insertion or modification

Nuclease-induced genome editing

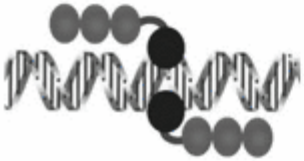


Different classes of endonuclease used for genome emgineering purposes

Meganucleases
(homing endonucleases)

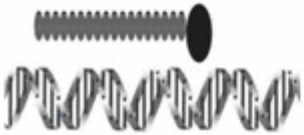
- Natural proteins
- 1st endonucleases used for genome engineering
- Low apparent modularity (2 separable domains)
- no separable catalytic domain

Zinc-Finger Nucleases



- Artificial protein : zinc finger protein (DNA binding domain) fused with a catalytic domain (FokI)
- 1st engineered endonuclease used to edit a human gene
- High modularity (6-8 separable domains "polydactyls")

Chemical endonucleases

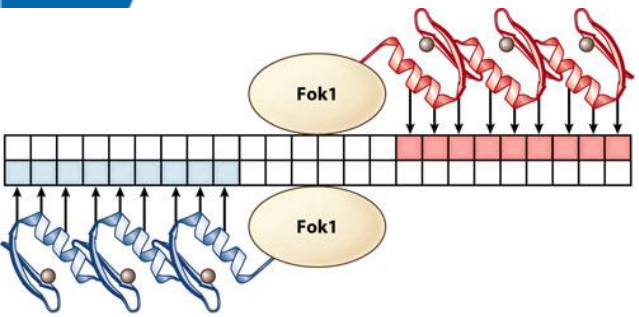


- Chemical DNA binding domain (TFO, polyamine) fused to effector (chemical or restriction enzyme)
- High modularity

TALE Nucleases

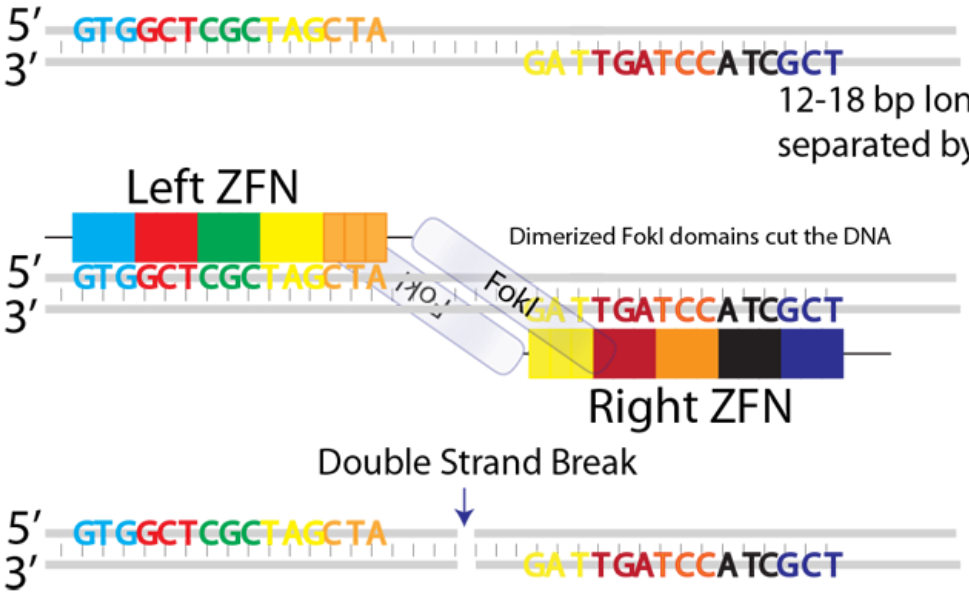
- DNA binding domain from Transcription Activator Like effectors from *Xanthomonas* fused to a catalytic domain
- Very high modularity
- simple Protein-DNA interaction (code)
- Early stage technology

Zinc-finger nucleases (ZFNs)

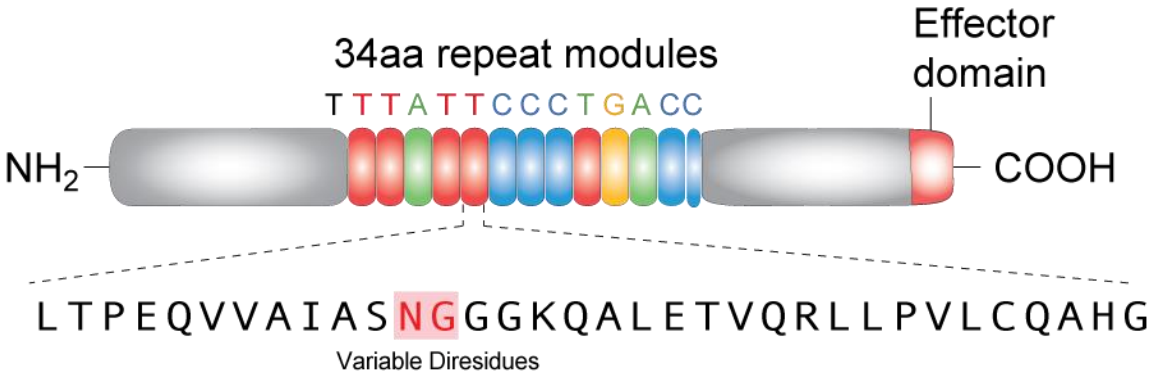


Klug A. 2010.
Annu. Rev. Biochem. 79:213–31

ZFN modules recognize triplets

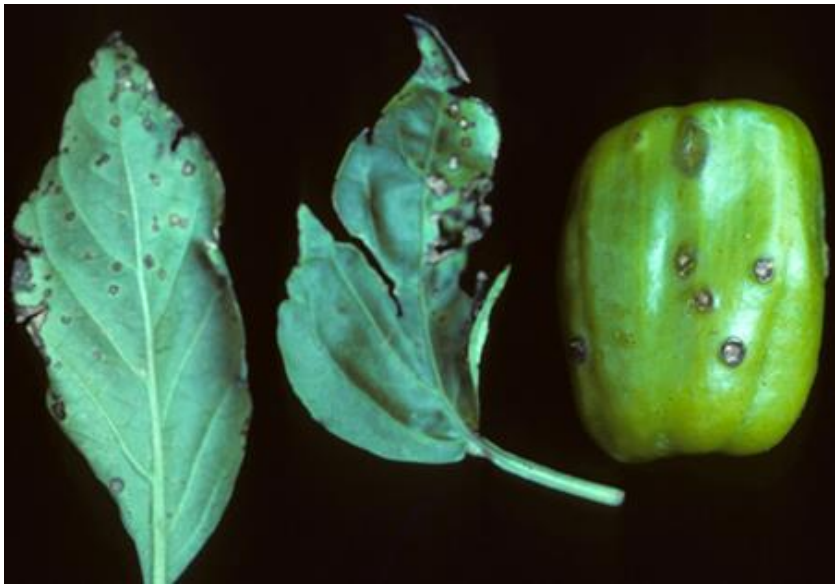


Transcription Activator-Like Effector



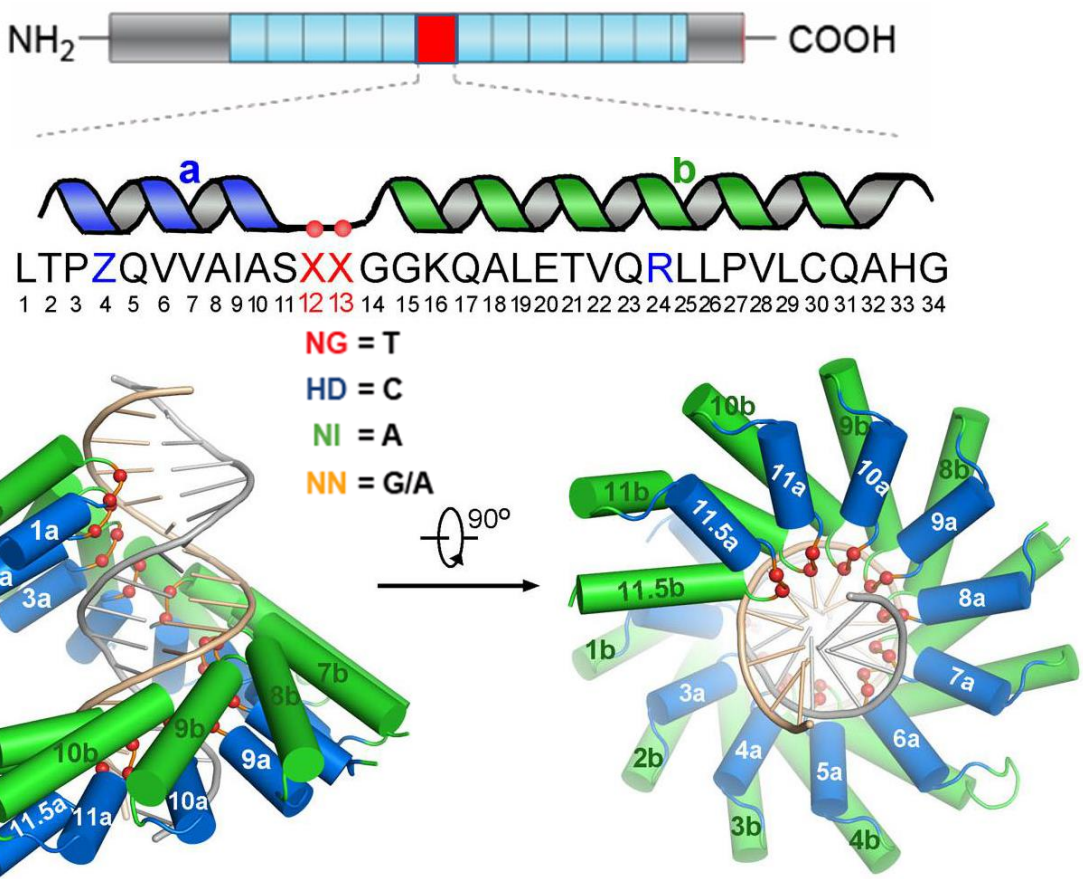
NG = T
HD = C
NI = A
NN = G or A

Bacterial spots induced by *Xanthomonas campestris* in plants

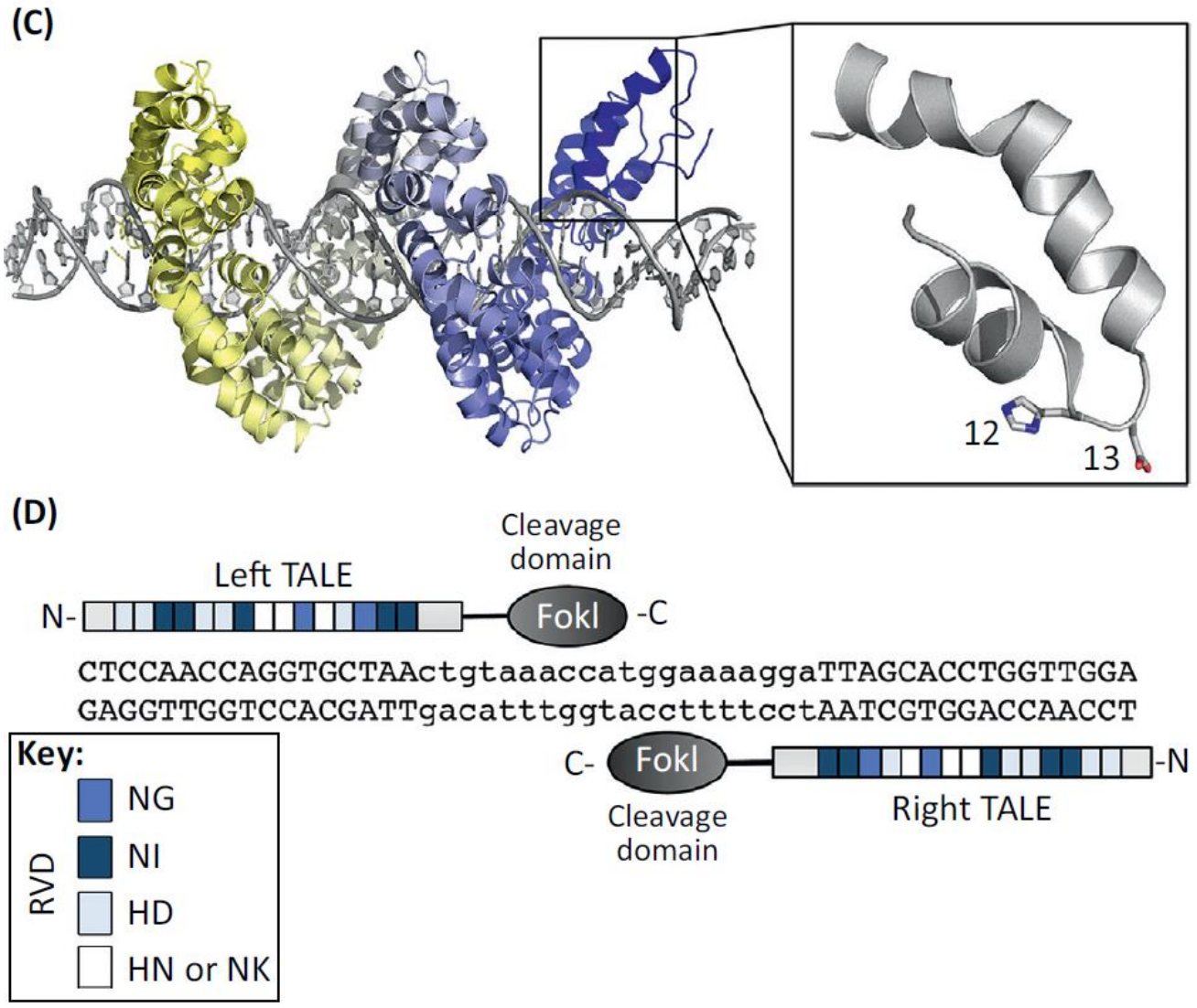


The Structure of TALE

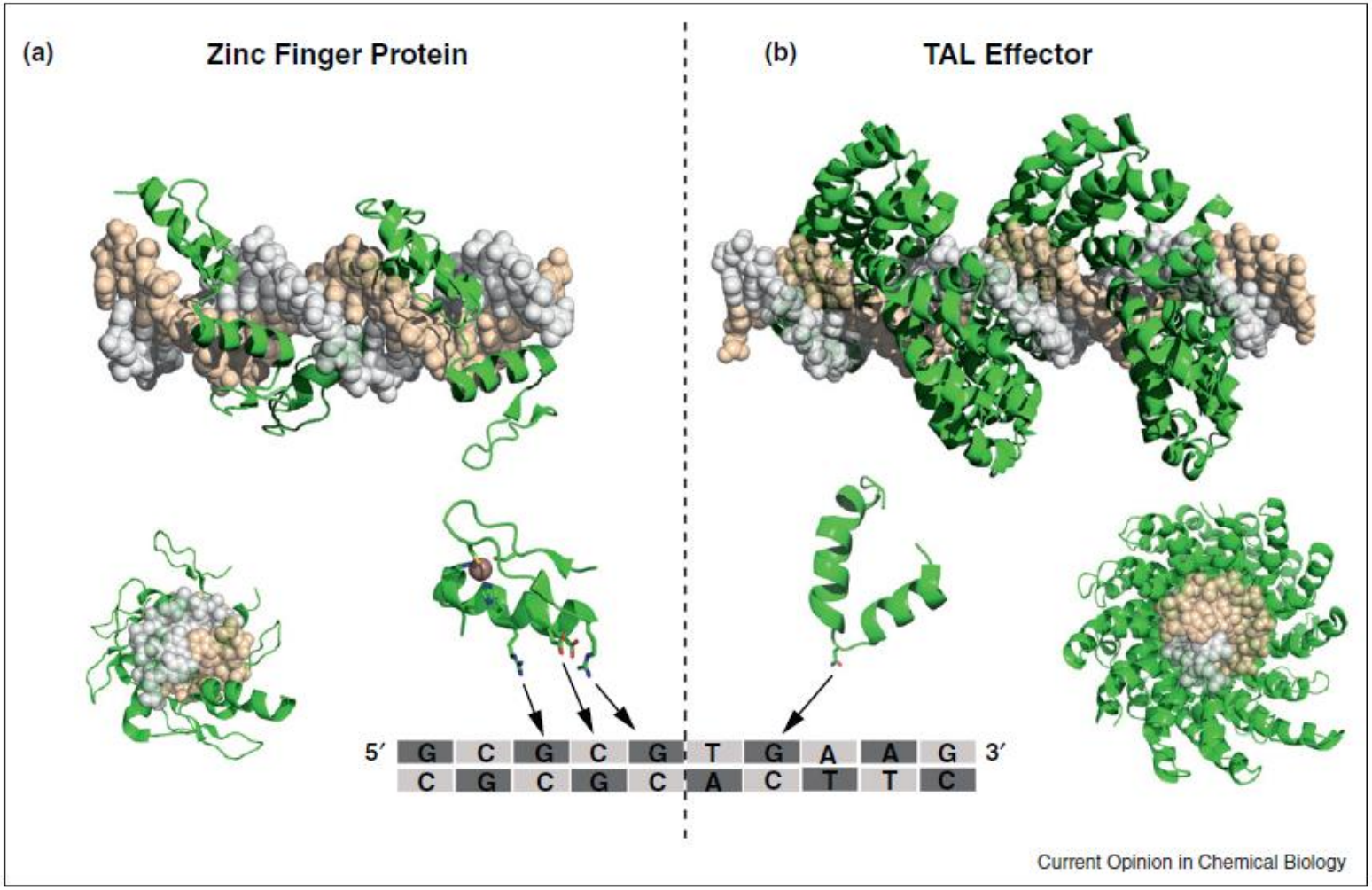
Repeat Variable Di-residues (RVD): 34 aa



TALEN: TAL effector nucleases



ZFN and TALEN DNA binding mechanisms



The applications of TALEN in different species

Knockout mice created by TALEN-mediated gene targeting (Nature Biotechnology, 2013)

Generation of *Rag1*-knockout immunodeficient rats and mice using engineered meganucleases (FASEB J, 2012)

Efficient targeted gene disruption in *Xenopus* embryos using engineered transcription activator-like effetor nucleases (TALENs) (PNAS, 2012)

In vivo genome editing using a high-effeciency TALEN system (Nature, 2012)

Efficient and specific modifications of the *Drosophila* genome by means of an easy TALEN strategy (JGG, 2012)

Transcription Activator-Like Effector Nucleases Enable Efficient Plant Genome Engineering (Plant Physiol., 2013)

High-efficiency TALEN-based gene editing produces disease-resistant rice (Nature Biotechnology, 2012)

Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting (Nucleic Acids Res., 2011)

Our TALEN Service

2011年起至今
力鈞生技已合成超過500組 TALEN pairs

劃時代的Gene Knockout技術
TALEN 靶向基因操作

目前TALEN技術已經成功應用於**植物、水稻基因修飾、阿拉伯芥、玉米、細胞、iPS、HIV治療、酵母、果蠅、斑馬魚及大、小鼠、剔除鼠、剔除豬**等各類研究動植物研究領域，替科學研究人員在研究基因功能上帶來前所未有的大躍進。

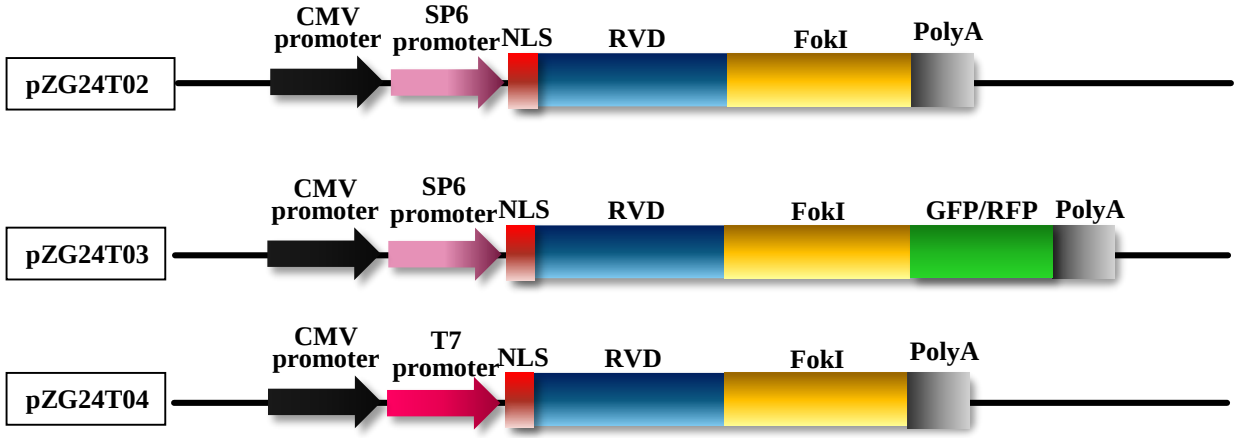
ZGENEBIO TALEN組裝服務特點：

1. 採用最新高通量合成技術、合成組裝速度世界第一快**20個工作天。**
2. 價位合理、以服務台灣研究社群為最高原則,**只需要國外1/2價格。**
3. 幾乎能對各種物種、動物、植物、細胞的標的基因序列進行剔除。
4. 篩選出突變細胞(體)的時間短、服務過的客戶都很滿意。
5. 基因剔除成功率高(約在70-80%左右)。
6. 基因剔除專一性高、毒性低、容易篩選出突變體。
7. 提供免費且專業的靶點預測服務。
8. 提供高效率改造**第三代載體**，為第一代載體**3-6倍效率**。
9. 提供最新改造 **GFP / RFP 螢光載體**方便篩選。

ZGene TALEN Genome Editing System

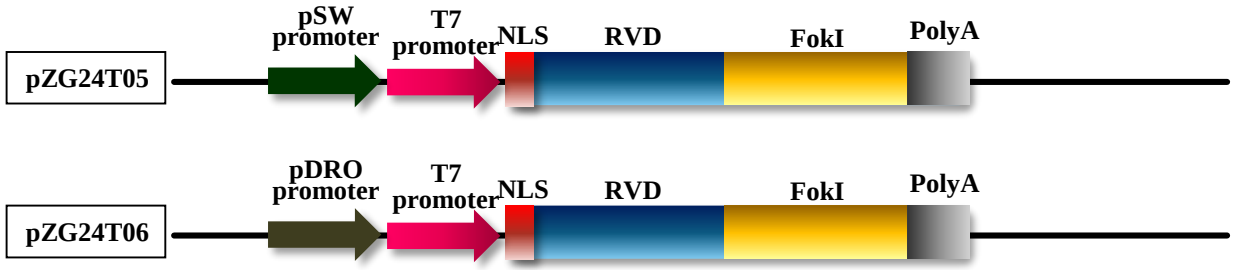
For Embryonic Stem Cells / Primary Cells / In Vitro Transcription

For Mammalian Cells / Tumor Cells / In Vitro Transcription



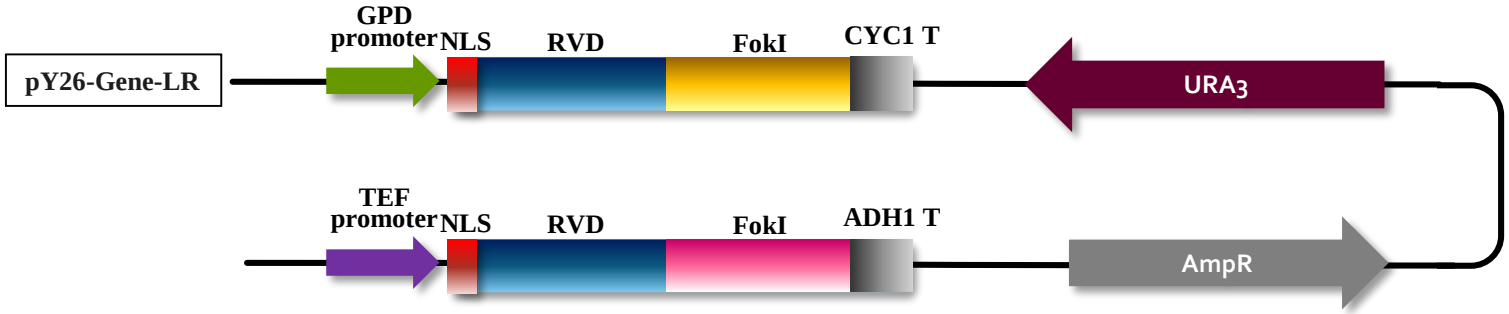
ZGene TALEN Genome Editing System

For Insect Cells / Egg / In Vitro Transcription

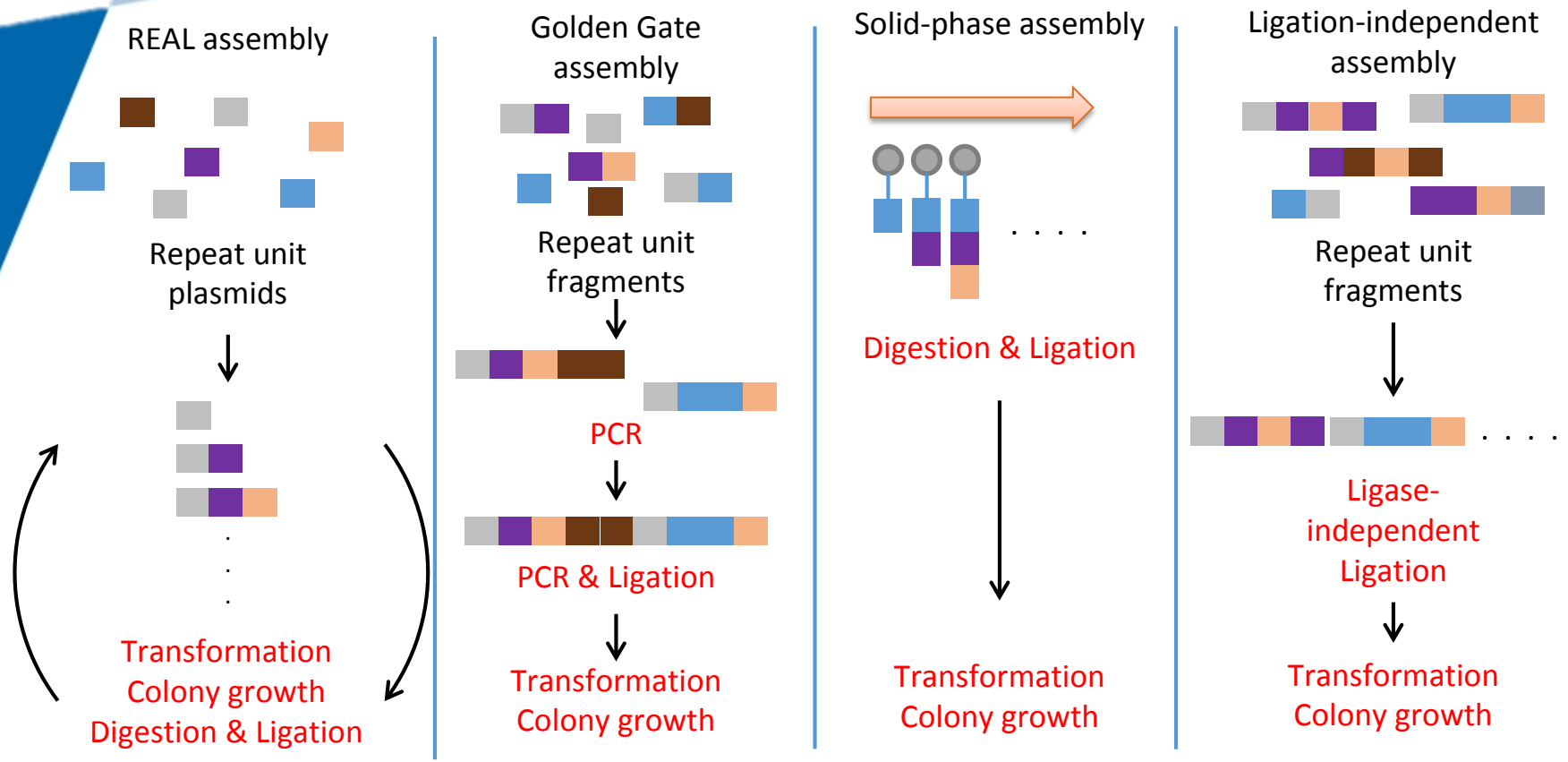


For Plants

For Yeast



Assembly technologies of TALEN

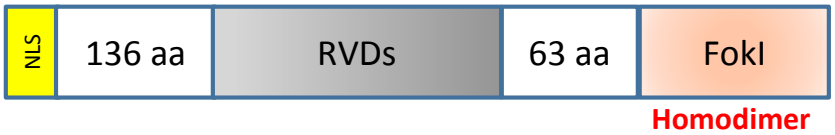
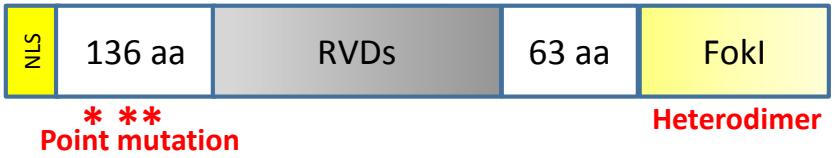


Assembly **cost**: Solid-phase > Ligation-independent ≥ Golden Gate ≥ REAL

Assembly **speed**: Solid-phase > Ligation-independent > Golden Gate > REAL

Assembly **successful rate**: Solid-phase ≥ Ligation-independent ≥ Golden Gate ≥ REAL

The 3rd generation ZGene eTALEN

Vector backbone	Relative activity
1st generation vector (Native form)	
	1
2nd generation vector (Goldy TALEN)	
	X 2 ↑
3rd generation vector (eTALEN)	
	X 6 ↑↑↑

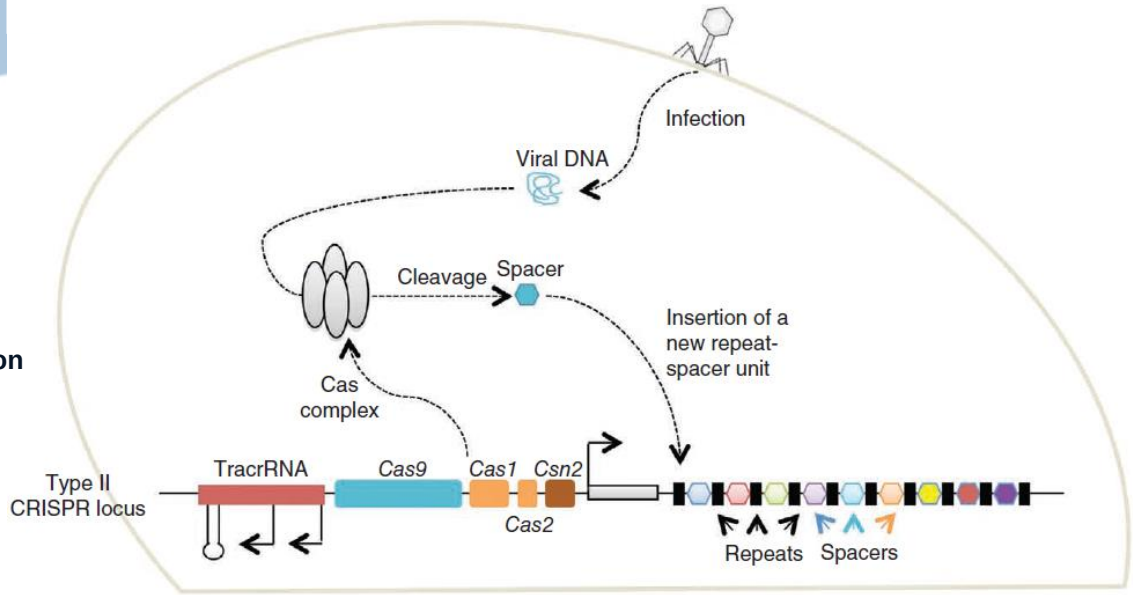
The activity of ZGene eTALEN is sixfold greater than NativeTALEN.

CRISPR System

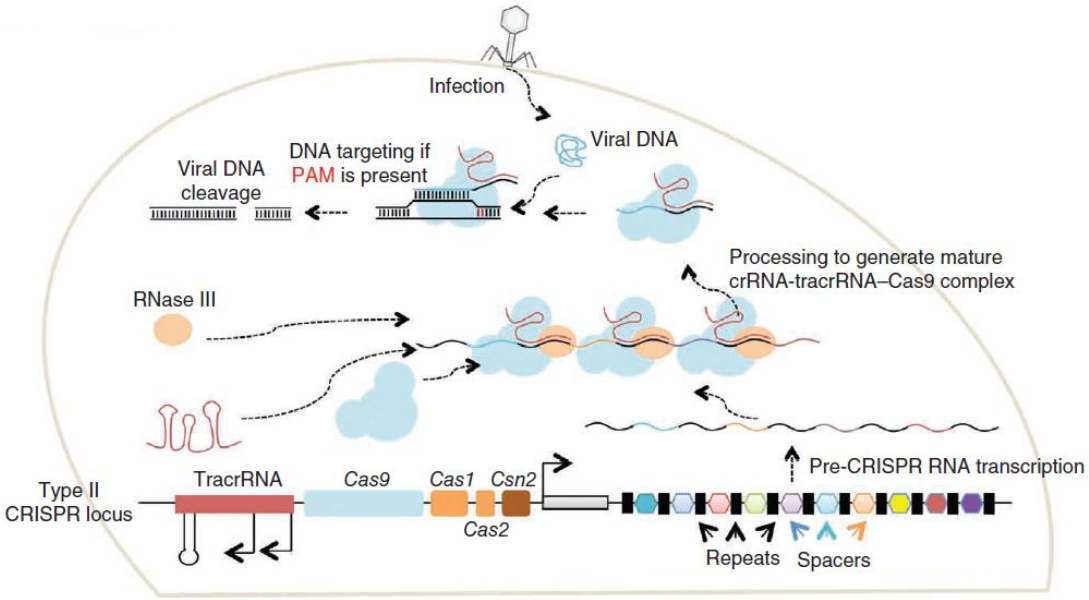
Background and Principle

Clustered regulatory interspaced short palindromic repeat (CRISPR)

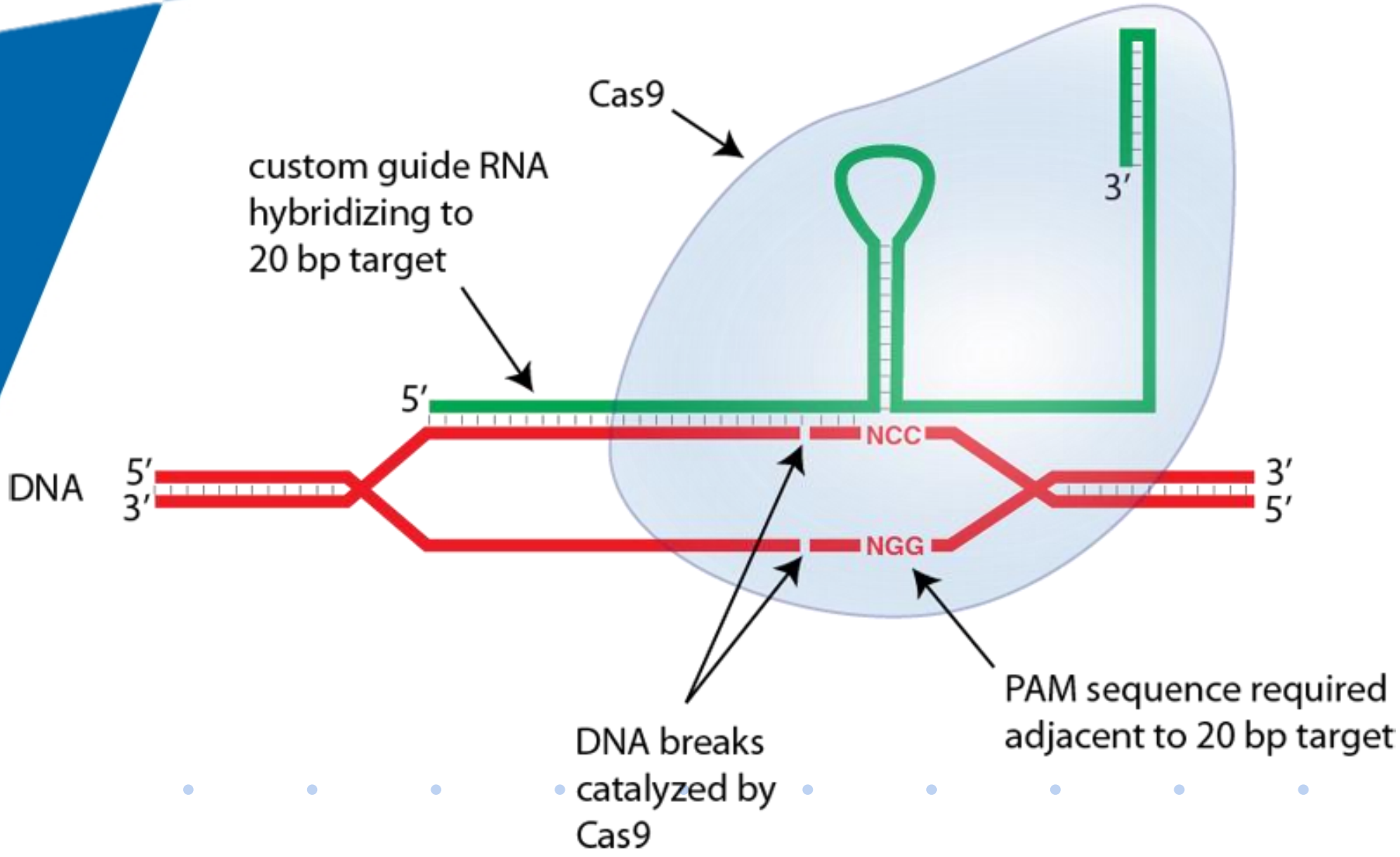
Phase1 : immunization



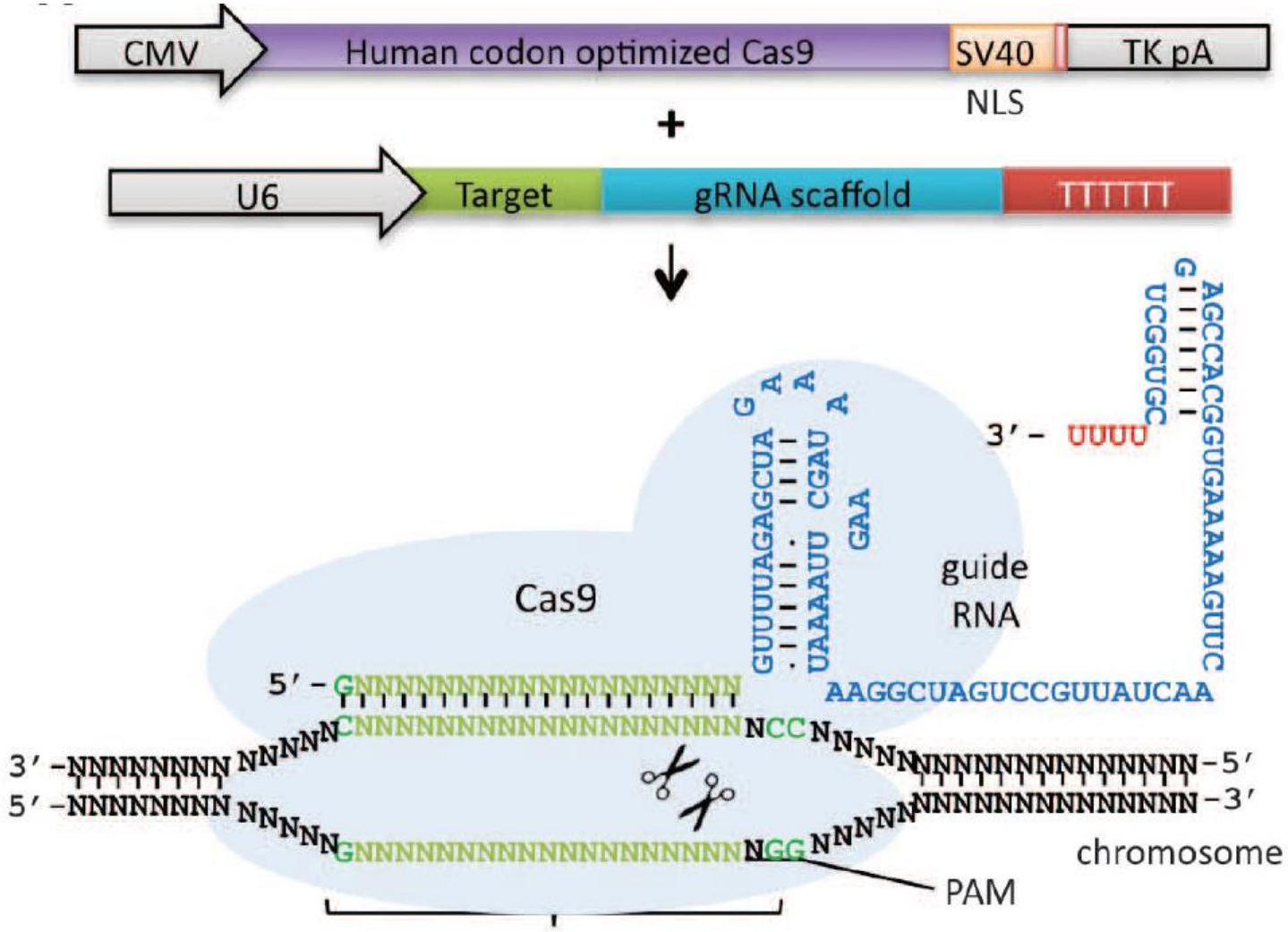
Phase2 : immunity



Clustered regulatory interspaced short palindromic repeat (CRISPR)



CRISPR-Cas9 system components

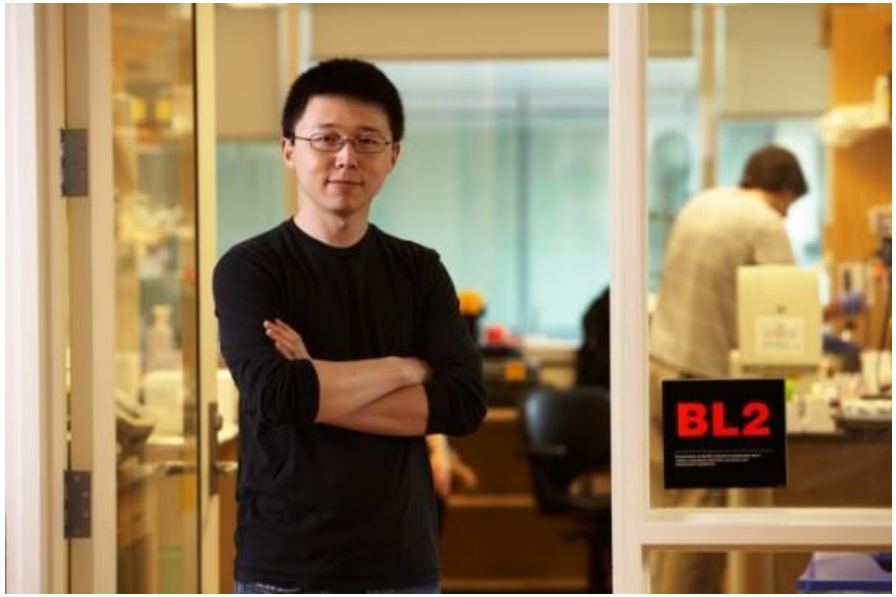


Multiplex Genome Engineering Using CRISPR/Cas Systems

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.

Nature 2013 TOP 10 Science



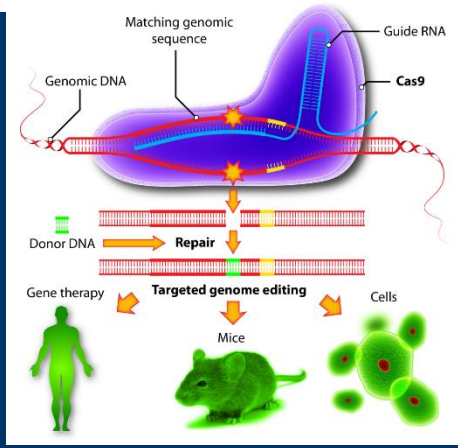
FENG ZHANG: DNA's master editor

Borrowing from bacteria, a biologist helps to create a powerful tool for customizing DNA.

By Daniel Cressey

Relative characteristics of genome-editing tools

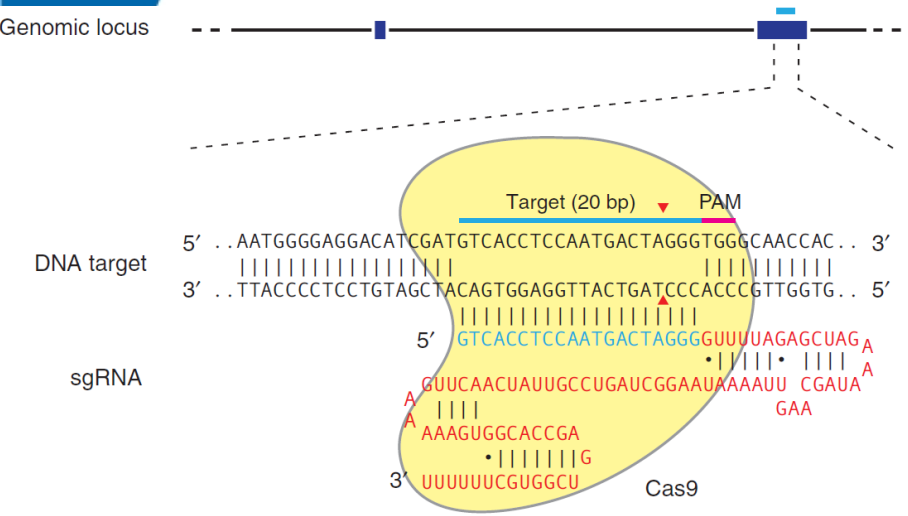
System	Origin	Typical genomic target site	Flexibility in site selection	Ease of use affordabillity	Efficay	Specificity lack of off-target effects
ZFNs	Adapted from zinc finger proteins widely found in nature	Pair of 9- or 12-bp sequences	+	+	++	++
TALENs	Adapted from TAL effector proteins in plant pathogens	Pair of 13-bp or longer sequences (no length limitation)	++	++	++	+++
CRISPR/ Cas	Adapted from bacterial (*S. pyogenes*) immune system	20-bp protospacer + 3-bp PAM (23- bp sequence)	++	+++	+++	+

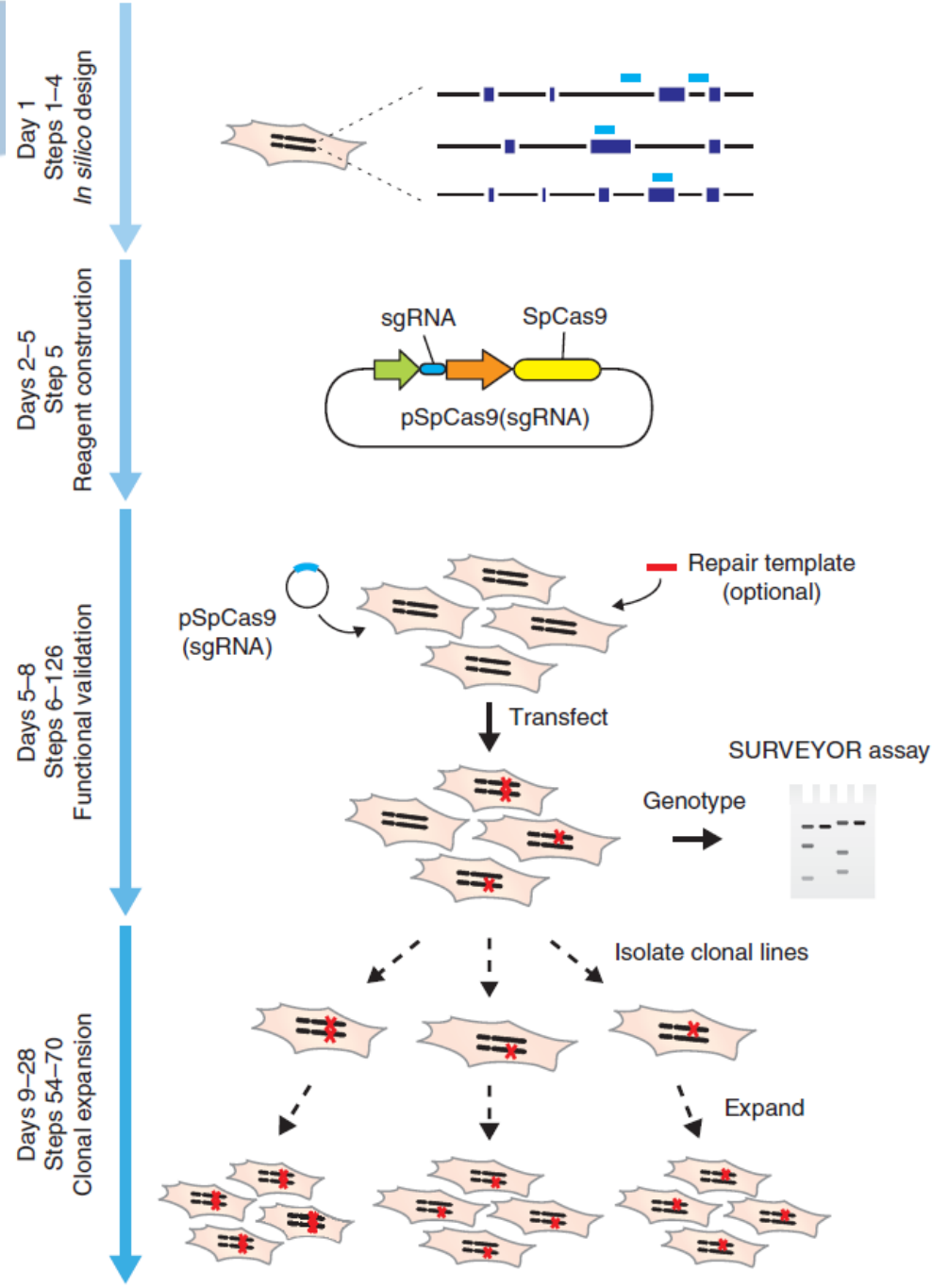


Application of CRISPR-Cas9

Mammalian Cells

CRISP-Cas9 system applied in cells

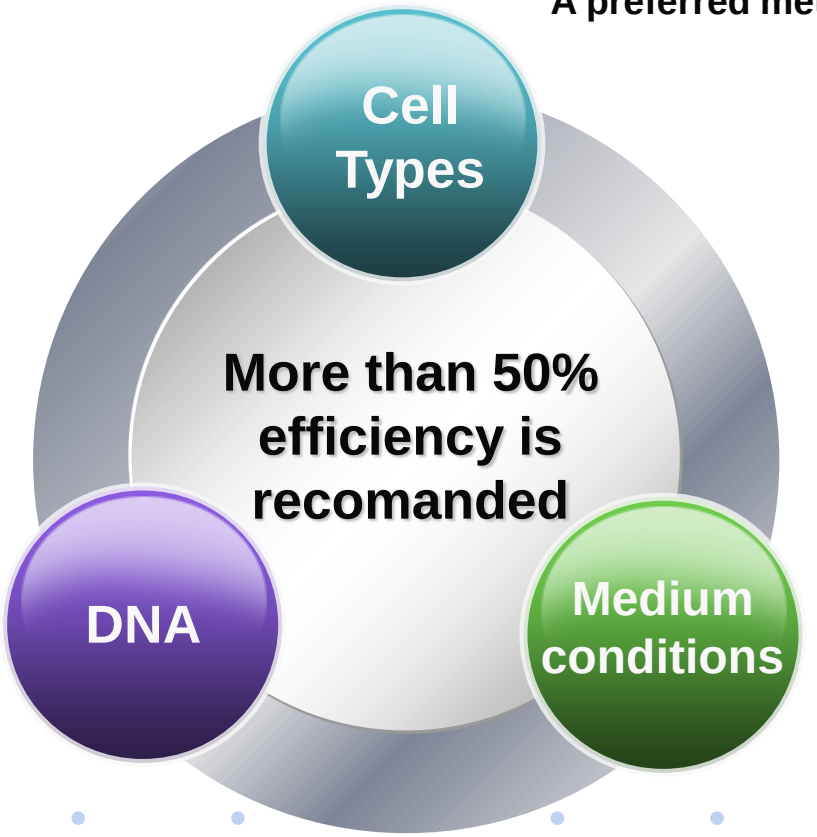




Transfection Efficiency

– **Transfection efficiency can be determined by antibiotic selection or fluorescence presentation**

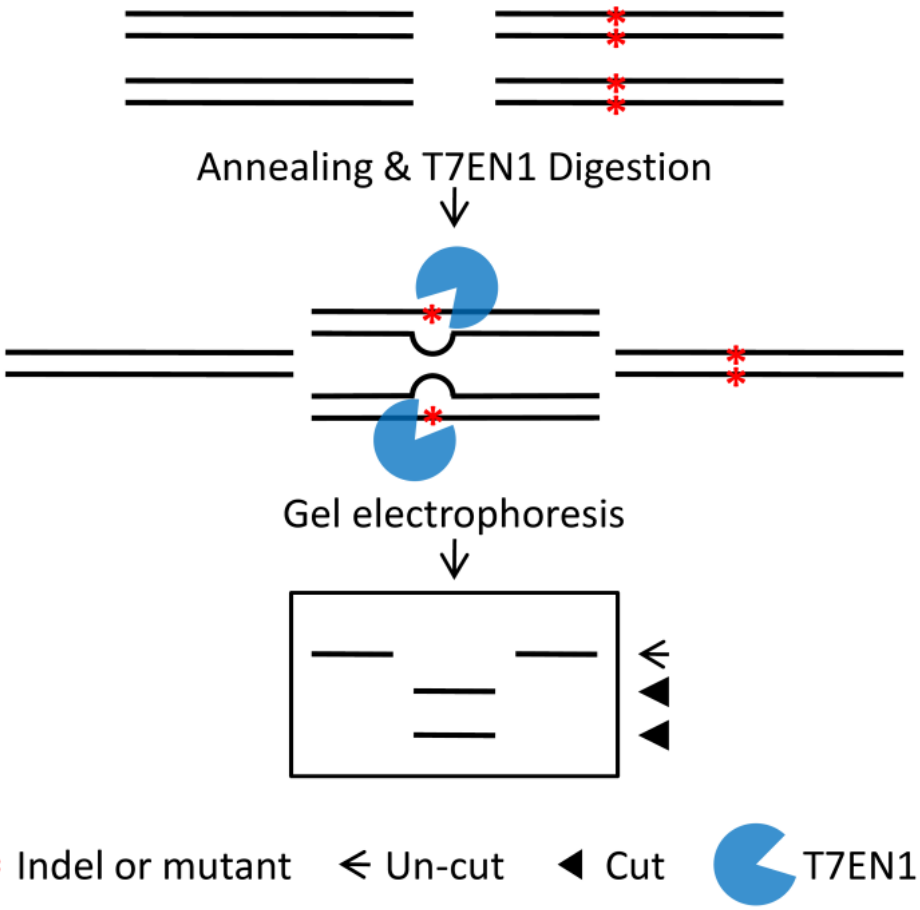
A preferred method in Lab was used



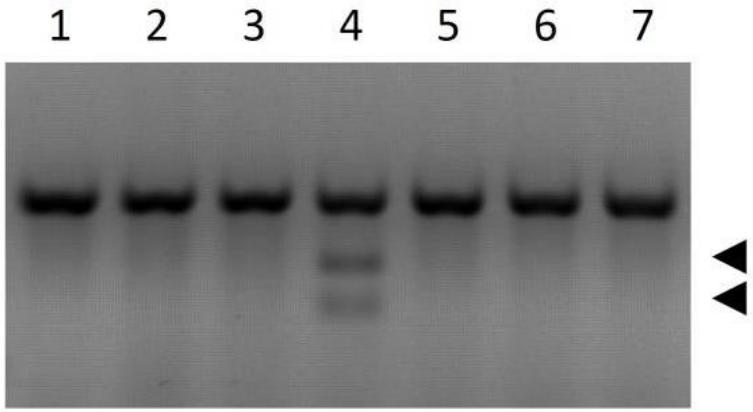
CRISPR plasmid is ~8-10K bp
Oligo DNA
ds DNA

Cell culture conditions

Mutation Screening



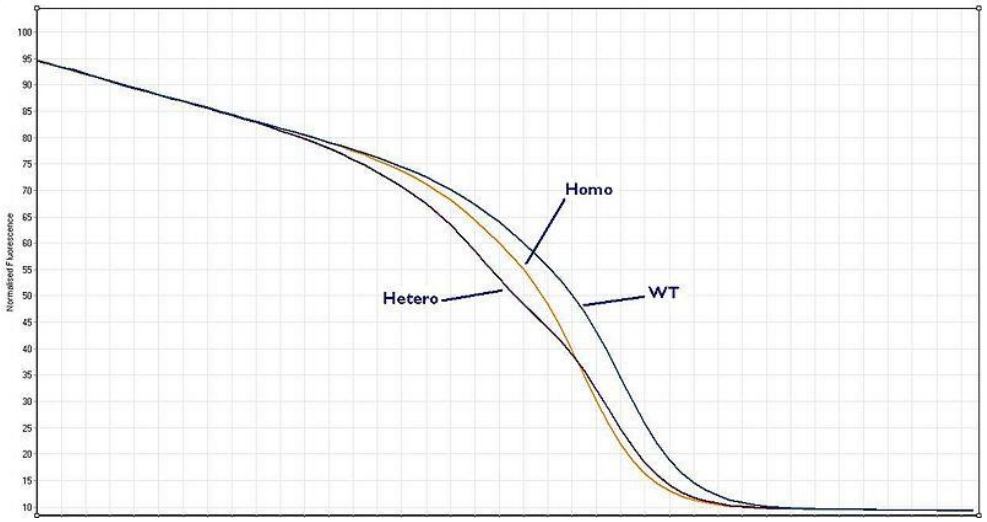
Mutation sequencing

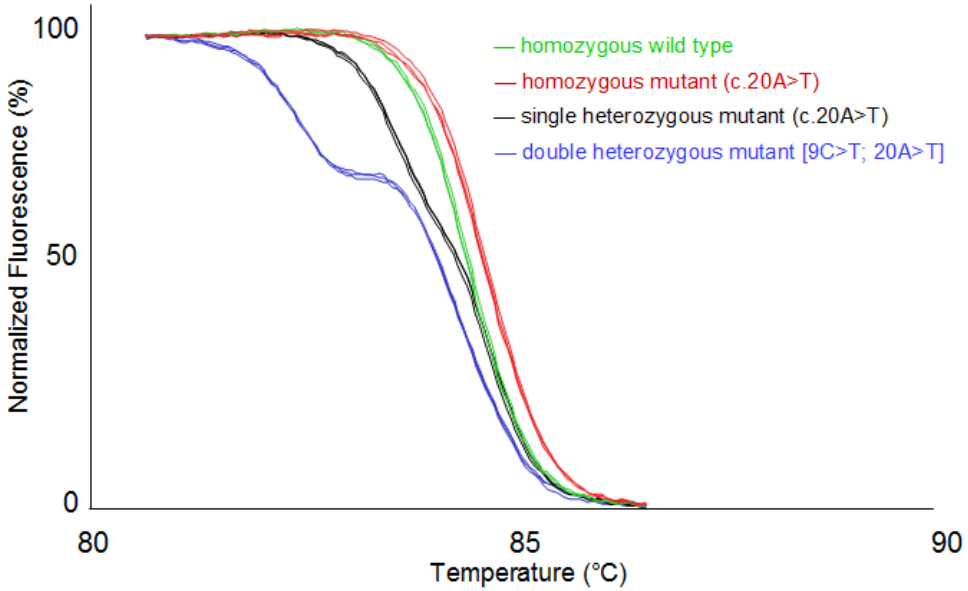


n=50

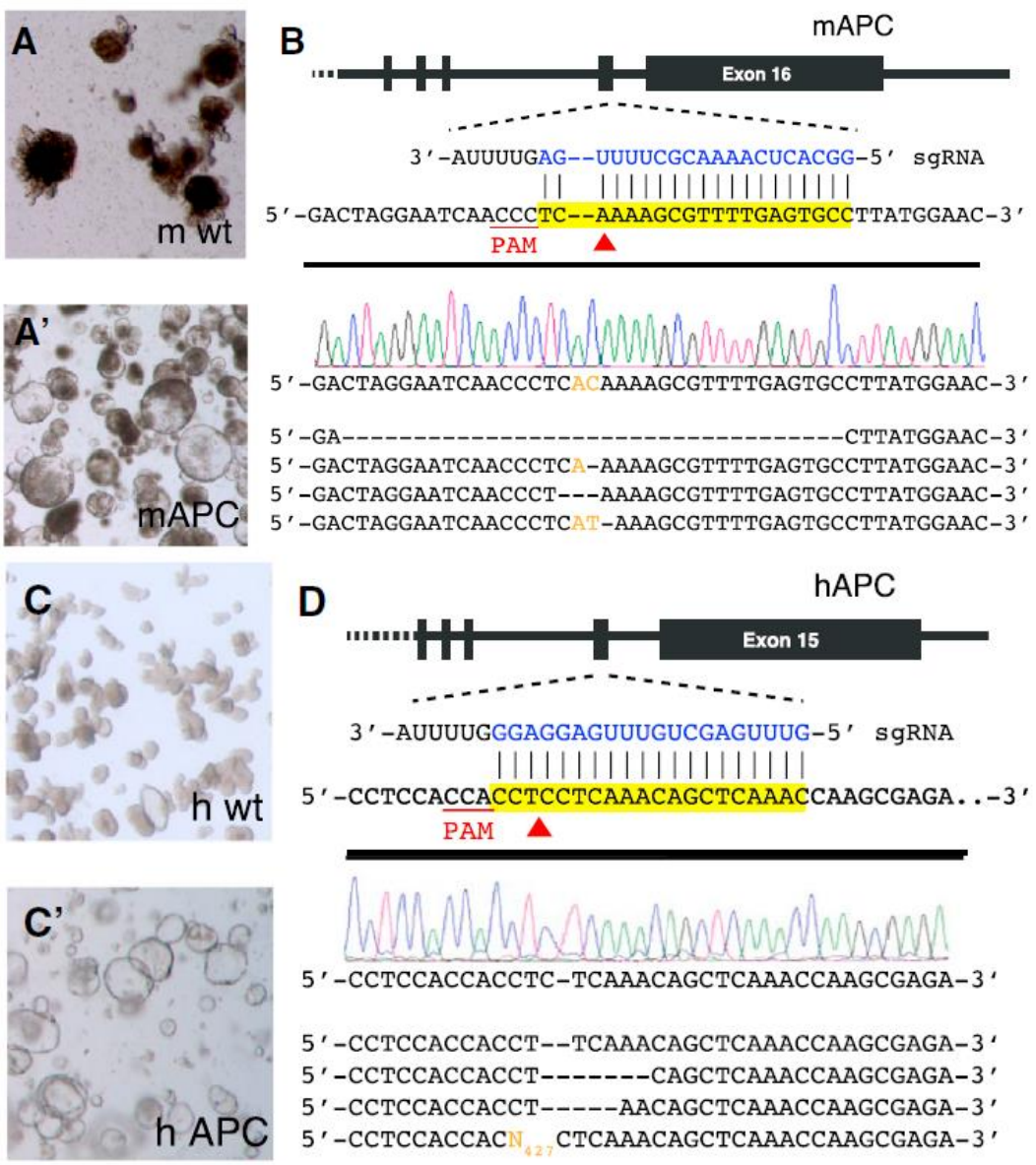
```
AGGCTACGTCCAGGAGCGCACCATCTTC:TTCAAGGACGACGGCAACTACAAGACC (WT)
AGGCTACGTCCAGGAGCGCACCATCTTCtTTCAAGGACGACGGCAACTACAAGACC (+1bp, x4)
AGGCTACGTCCAGGAGCGCACCATCTTC:TT:::GGACGACGGCAACTACAAGACC (-3bp)
AGGCTACGTCCAGGAGCGCACCATCTT::::::GGACGACGGCAACTACAAGACC (-6bp)
AGGCTACGTCCAGGAGCGCACCATCTTC:::::::GACGACGGCAACTACAAGACC (-6bp)
AGGCTACGTCCAGGAGCGCACCATCTTC::::AAGGACGACGGCAACTACAAGACC (-3bp)
AGGCTACGTCCAGGAGCGCACCATC:::::::AAGGACGACGGCAACTACAAGACC (-6bp, x3)
```

High Resolution Melting (HRM)

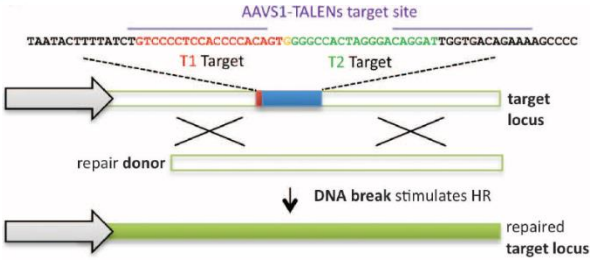


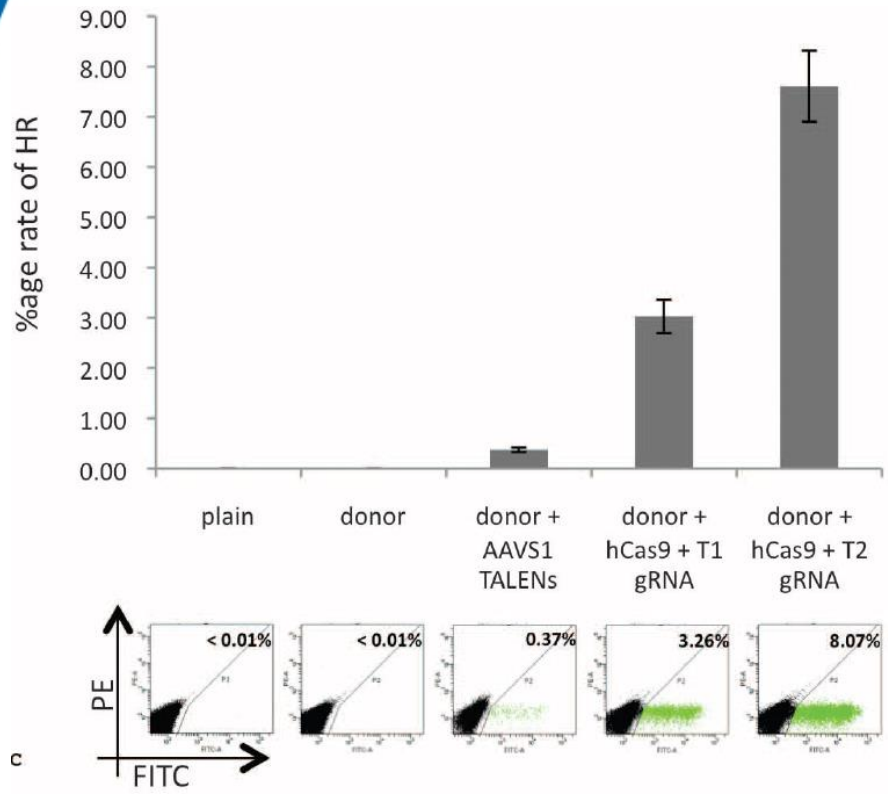


CRISPR/Cas9-Mediated Genome Editing in Adult Stem Cells



Targeting Rates of CRISPR/Cas System



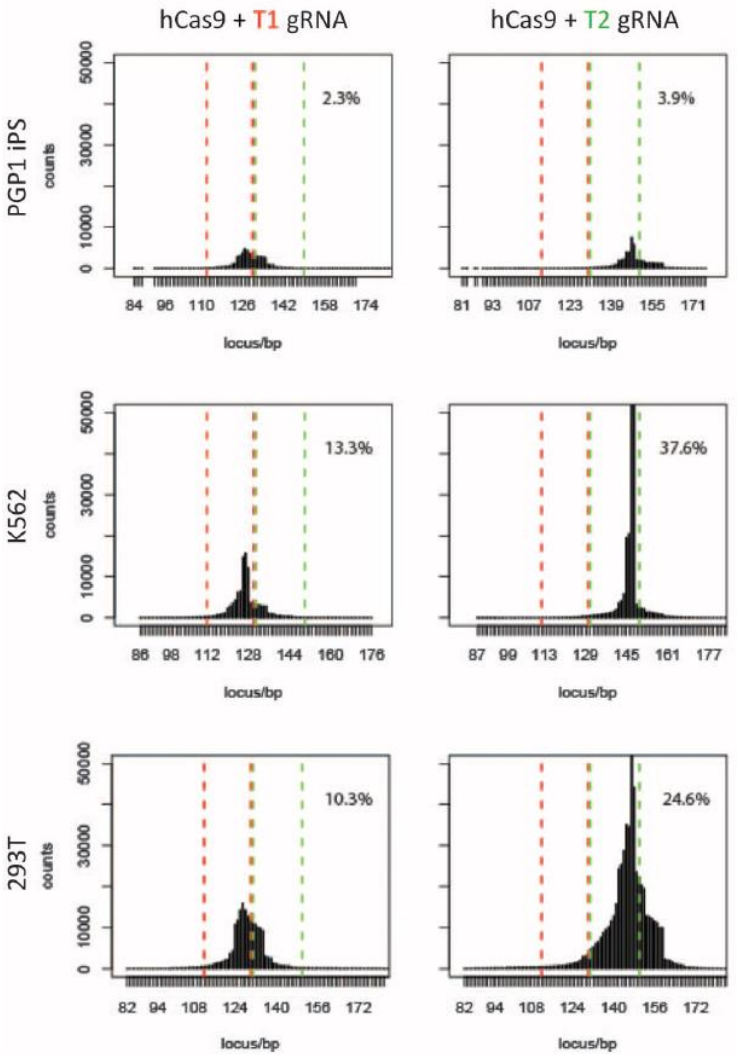


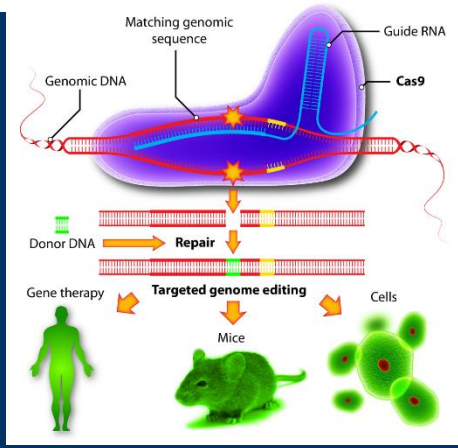
endogenous 'native' hAAVS1 locus sequence

..TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGA..

T1 target T2 target

NHEJ rates evaluated using NGS of targeted AAVS1 locus

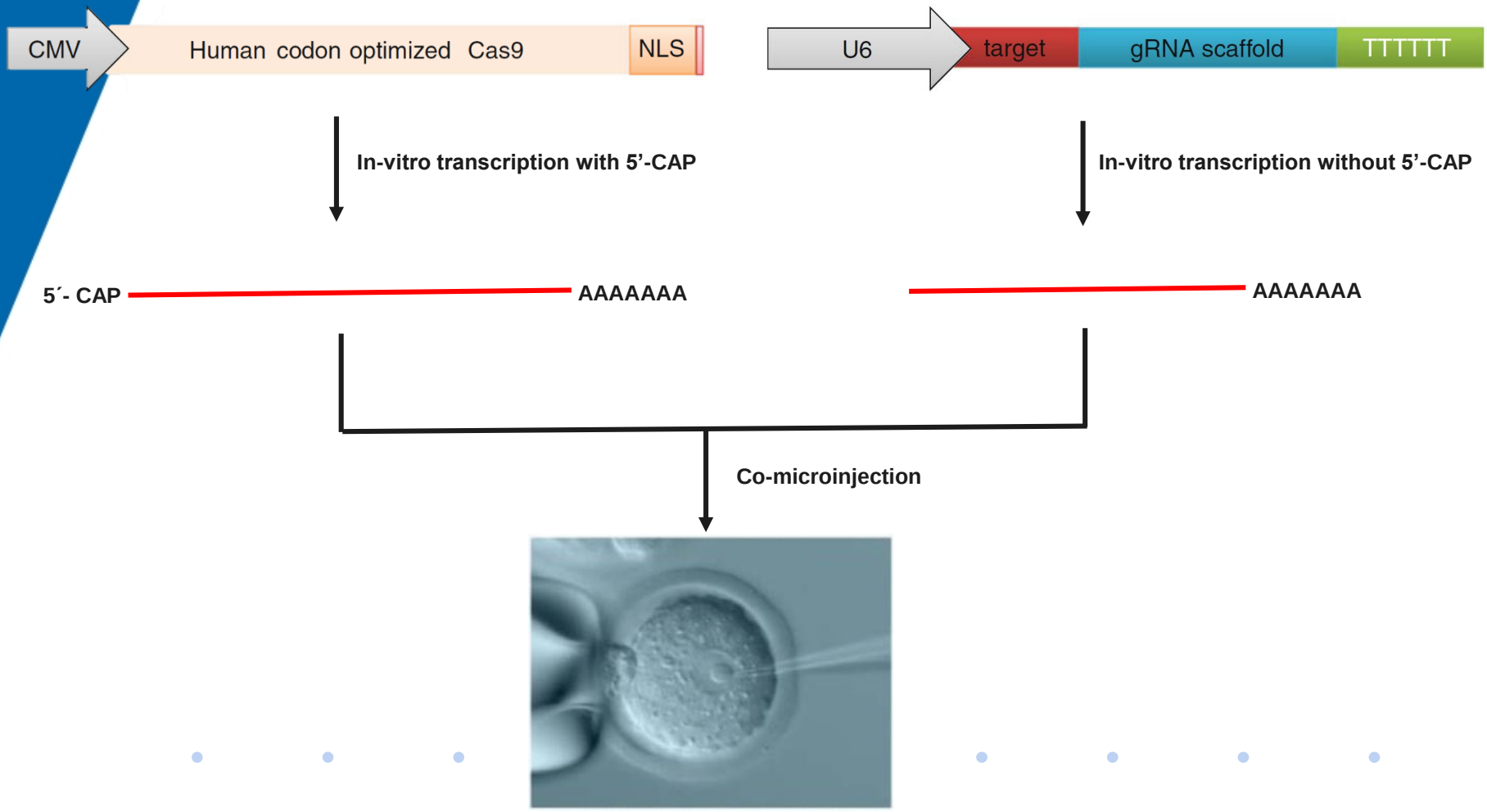




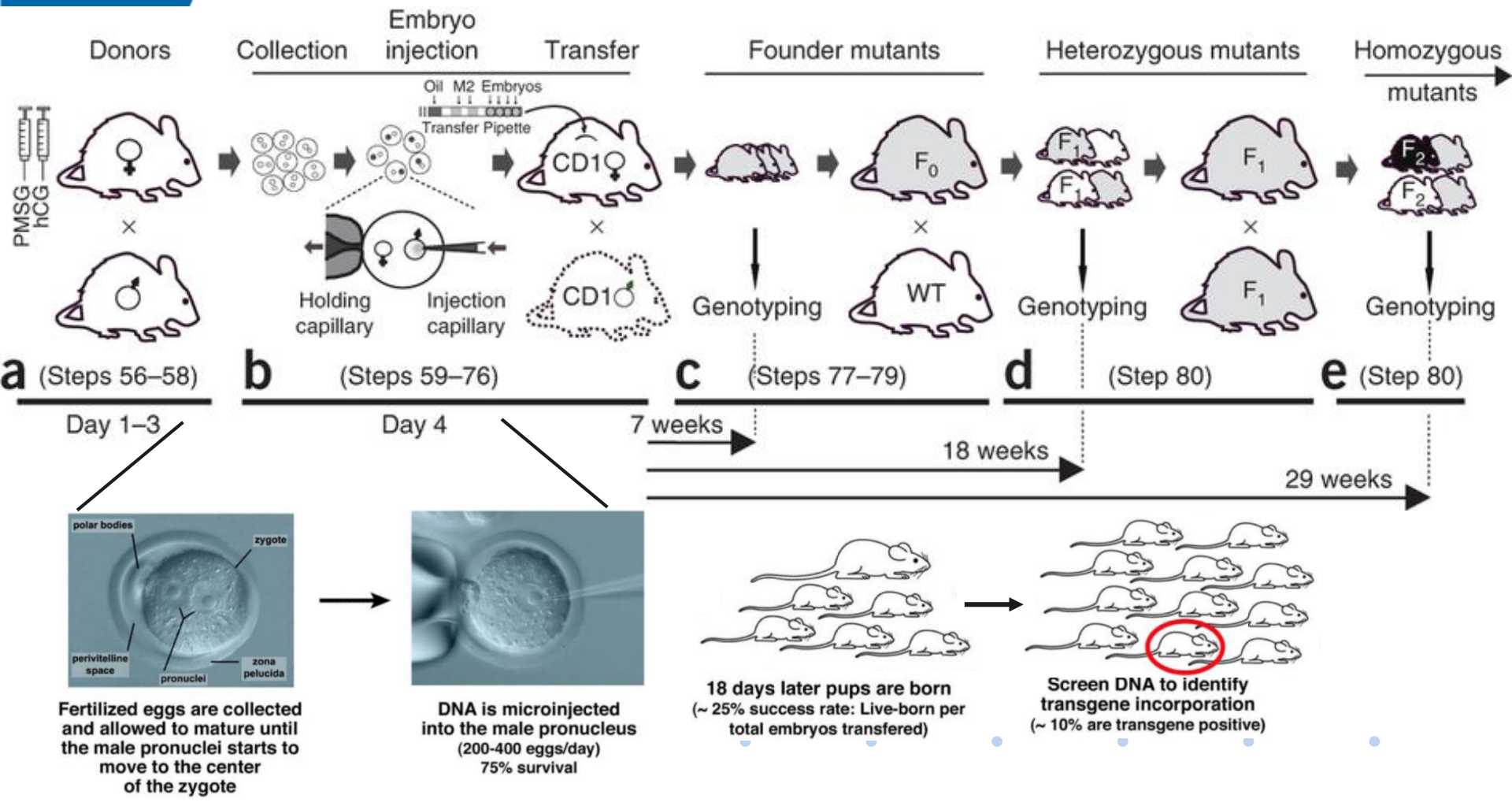
Application of CRISPR-Cas9

Transgenic Animals

In-vitro transcription of Cas9 and gRNA



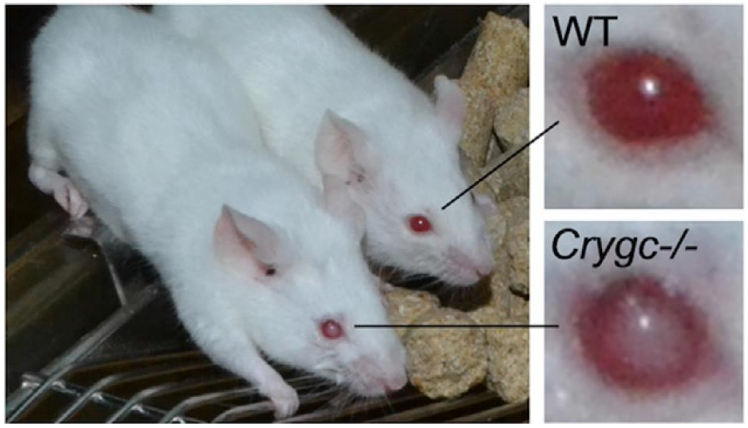
Generation of targeted mouse mutants by embryo microinjection

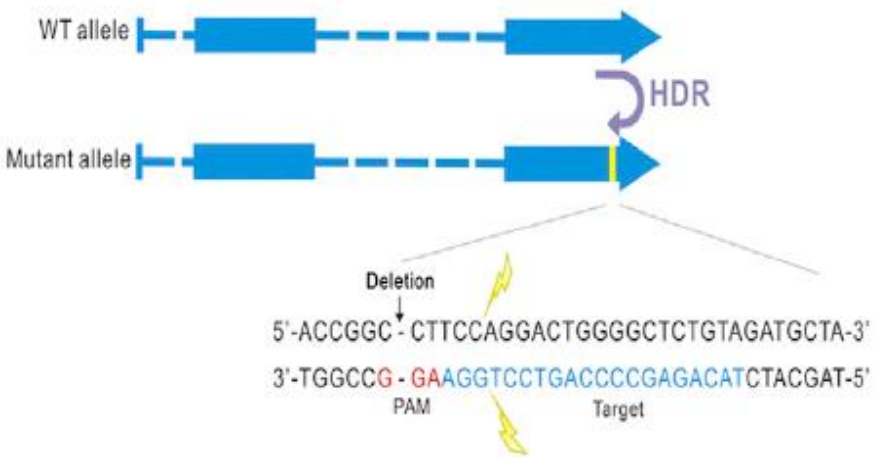


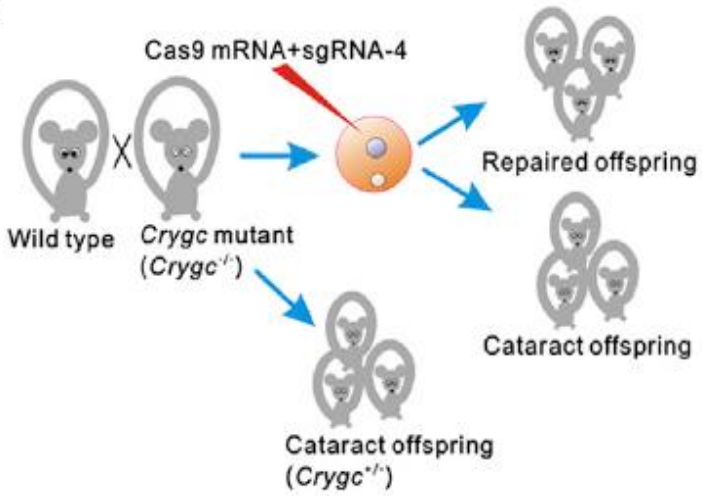
Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9

γ-crystallin

Crygc mutation (dominant inheritance)







Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9

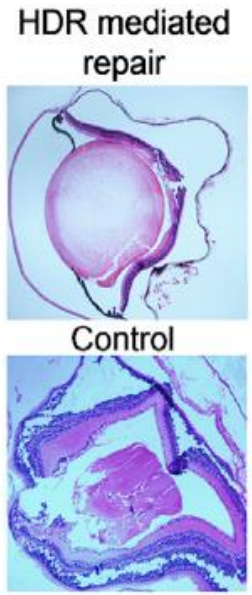


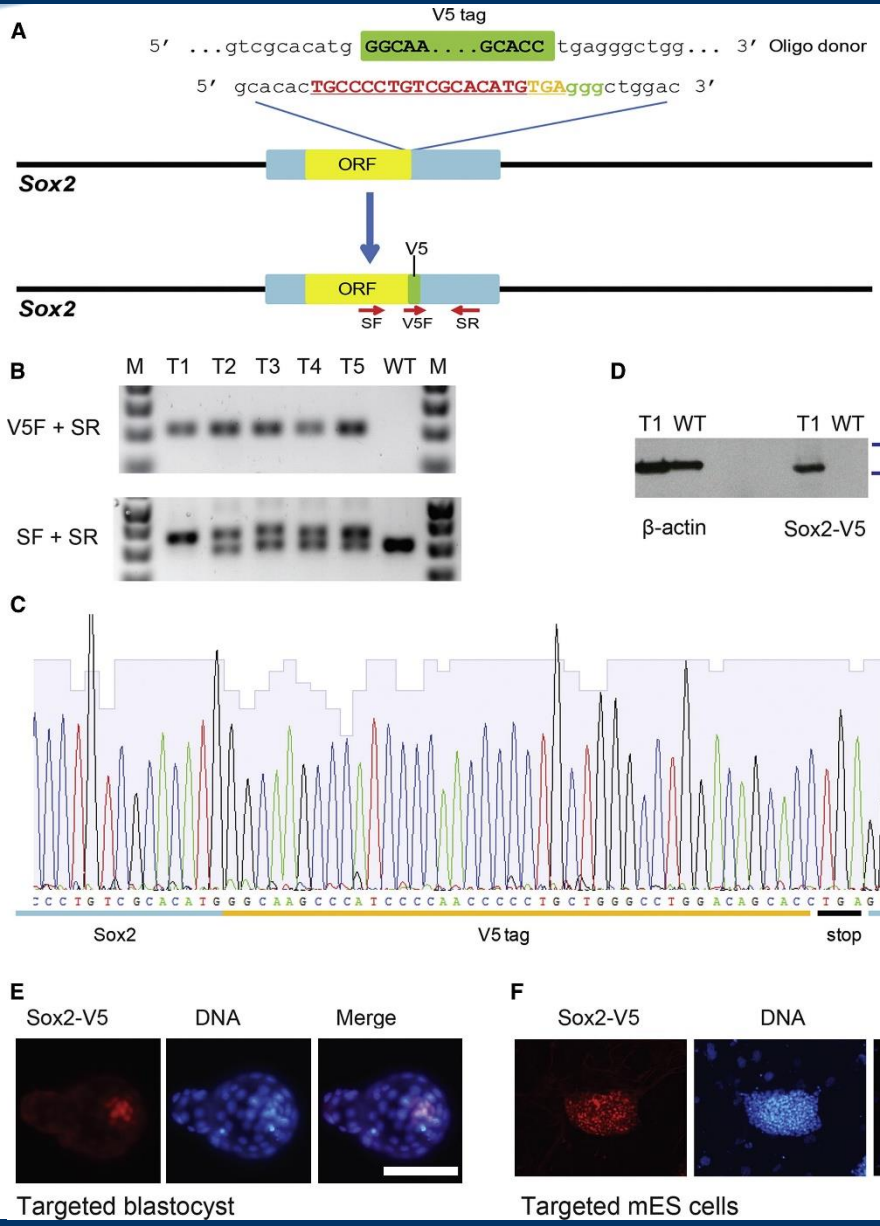
Table 1. CRISPR-Cas9-Mediated Gene Correction in Cataract Mice

Oligo	Injected Embryos	Blastocysts (Percentage of Injected Embryos)	Transferred Blastocysts	Live-Born Pups	Genetic Modification		NHEJ-Mediated Repair/Nonrepair	HDR-Mediated Repair
					WT allele	mutant allele		
-	172	157 (91%)	135	22	0	10	2/4	4
Oligo-1	245	213 (87%)	178	29	0	14	4/5	5
Oligo-2	221	190 (86%)	159	27	0	12	5/3	4

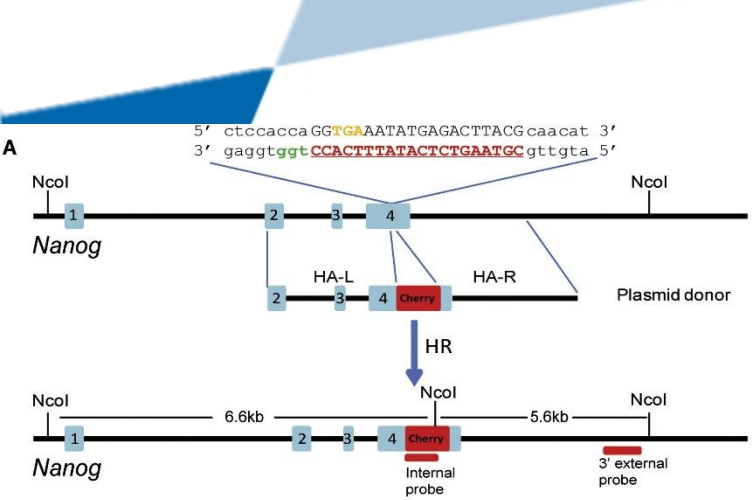
Cas9 mRNA and sgRNA-4 targeting mutant allele of *Crygc* gene were coinjected into fertilized oocytes with or without exogenous oligonucleotides (Oligo-1 or Oligo-2). The blastocysts derived from the injected embryos were transferred into uteri of pseudopregnant females. Newborn pups were obtained and genotyped. See also Figures 1, S1, and S2.

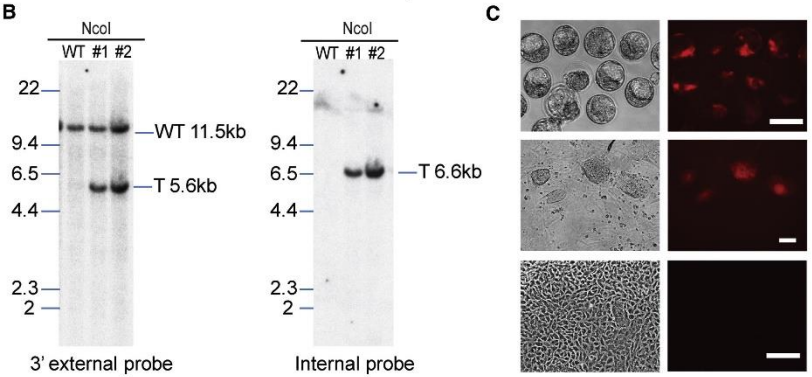
Mice with Reporters in the Endogenous Genes

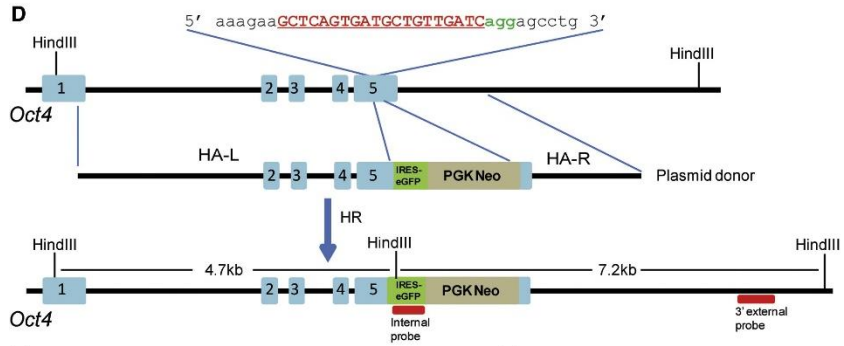
Zlastocysts/Injected Zygotes	Targeted Blastocysts /Total	Target ESCs /Total	Transferred embryos (recipients)	Knockin pre- and postnatal mice/Total
414/498	ND	7/16	200(10)	12/35

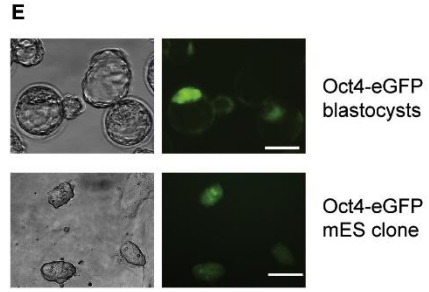


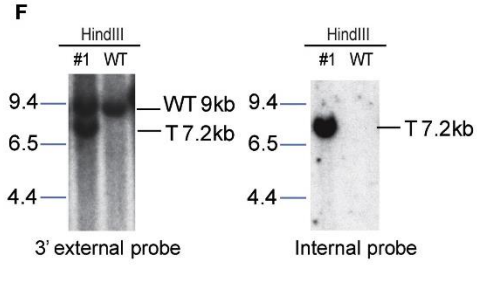
Mice with Reporters in the Endogenous Genes





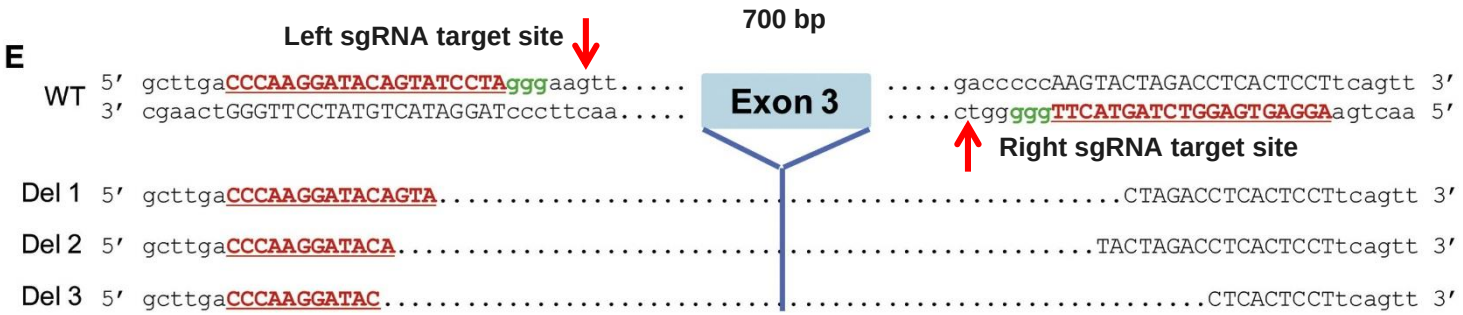




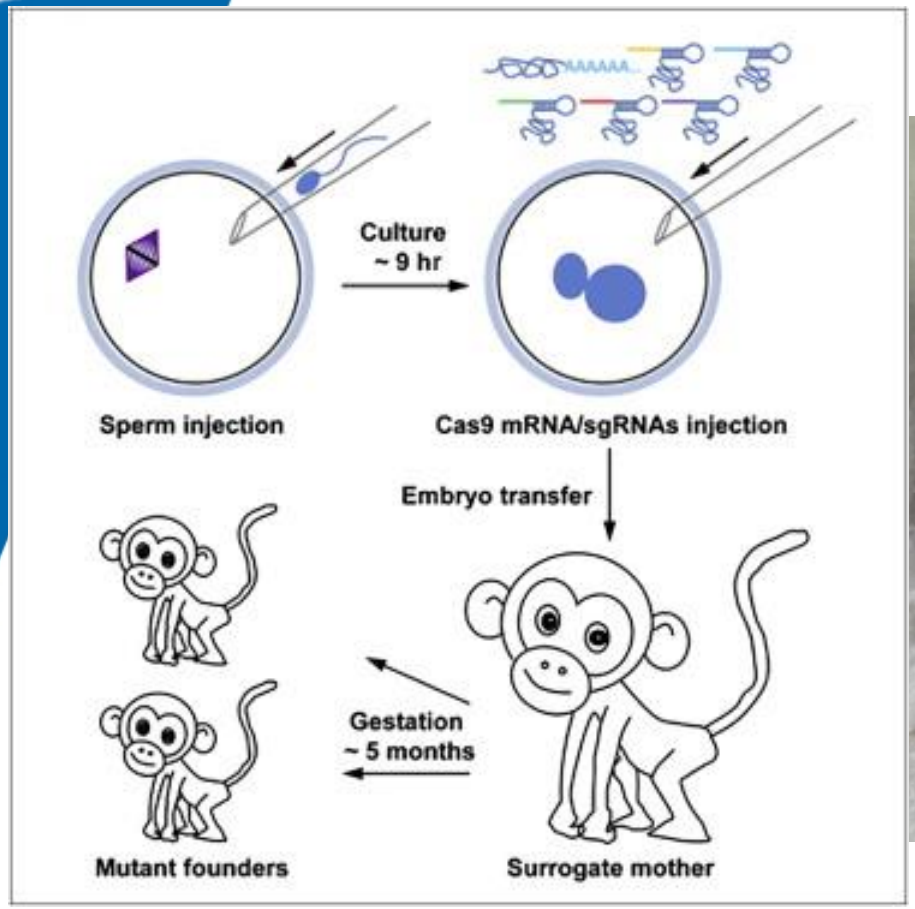


Donor	Zlastocysts/Injected Zygotes	Targeted Blastocysts/Total	Target ESCs /Total	Transferred embryos (recipients)	Knockin pre-and postnatal mice/Total
Naog-mCherry	936/1262	86/936	ND	415(21)	7/86
Oct4-GFP	254/345	47/254	3/9	100(4)	3/10

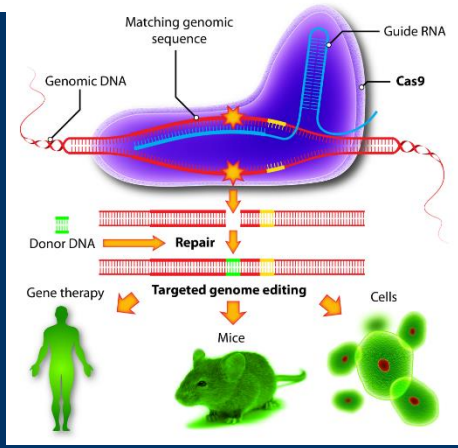
Injection of Cas9 mRNA and two sgRNA generated mutant allele with deletion of exon 3



Twin cynomolgus monkeys born in China are the first with mutations in specific target genes using the CRISPR/Cas9 system.



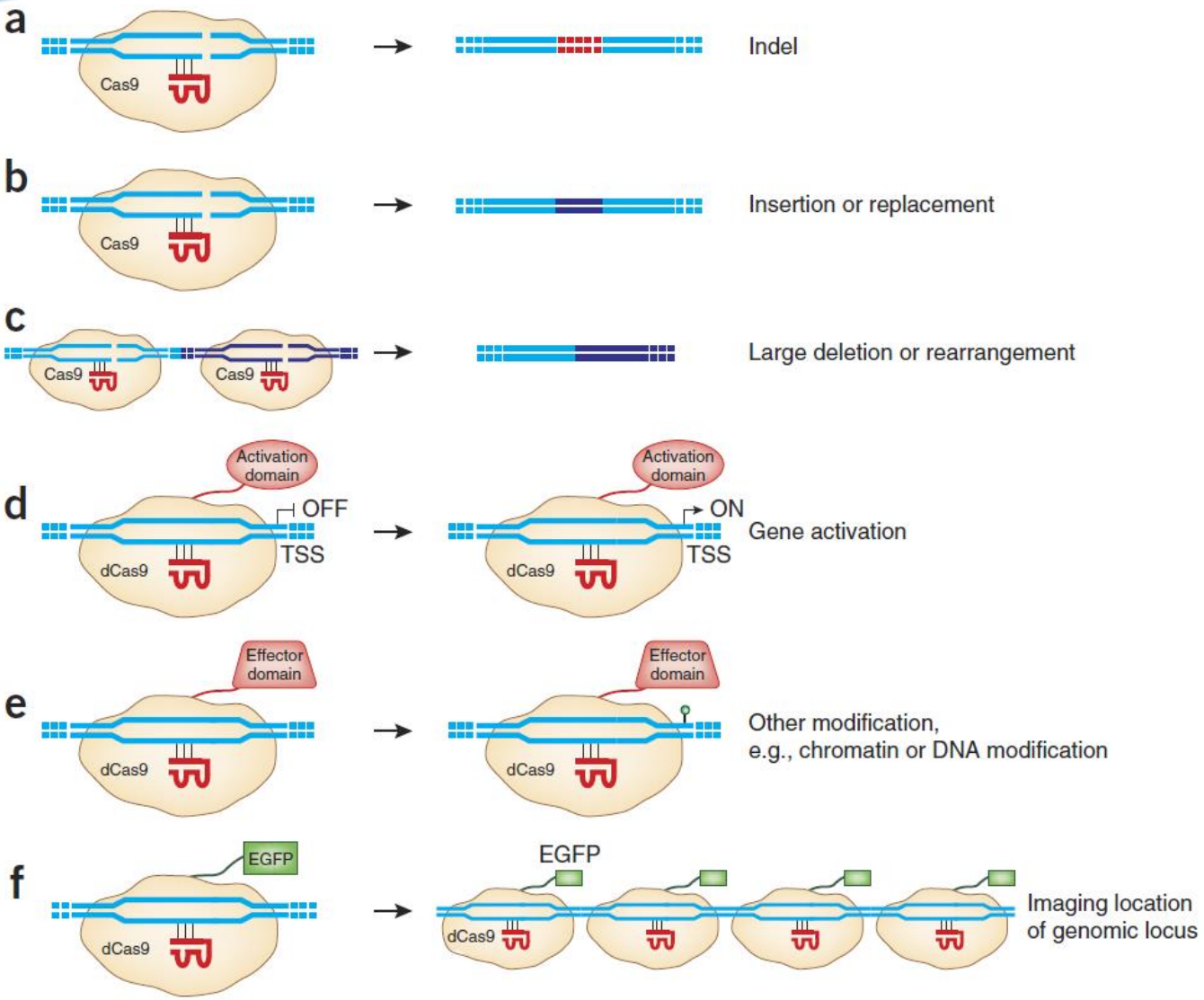
Two genes mutation:
***Ppar*-γ: helps to regulate metabolism**
***Rag1*: involved in healthy immune function**



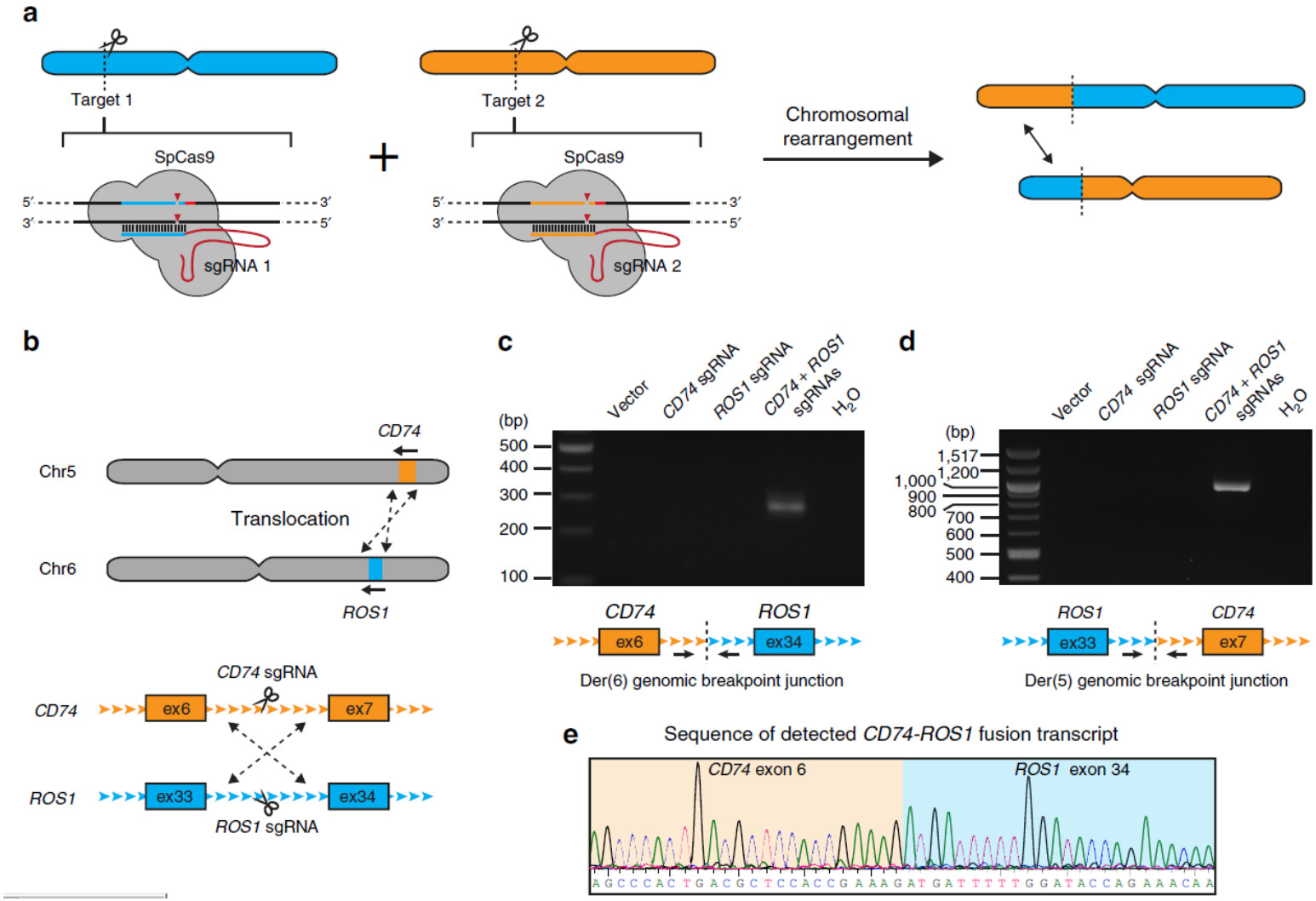
Application of CRISPR-Cas9

Others

Overview of various Cas9-based applications



Targeted genomic rearrangements using CRISPR/Cas technology



Published examples of cell types and organisms modified by Cas9

Cell type or organism	Cas9 form	Cell type	Reference numbers
Human cells	Cas9 nuclease	HEK293FT, HEK293T, HEK293, K562, iPSC, HUES9, HUES1, BJ-RiPS, HeLa, Jurkat, U2OS	9,13–16,47, 49–51,54,59, 84,85
	Cas9 nickase	HEK293FT, HEK293T	13,14,47,49
	dCas9 (gene regulation)	HEK293FT, HEK293T	70–72,74,82
	dCas9 (imaging)	HEK293T, UMUC3, HeLa	81
Mouse or mouse cells	Cas9 nuclease	Embryos	14,24–26
	Cas9 nickase	Embryos	47
	dCas9 (gene regulation)	NIH3T3	74
Rat	Cas9 nuclease	Embryos	26,36
Rabbit	Cas9 nuclease	Embryos	27
Frog	Cas9 nuclease	Embryos	28
Zebrafish	Cas9 nuclease	Embryos	17,33,37,60,85
Fruit fly	Cas9 nuclease	Embryos	29,30,61
Silkworm	Cas9 nuclease	Embryos	31
Roundworm	Cas9 nuclease	Adult gonads	32,62–67
Rice	Cas9 nuclease	Protoplasts, callus cells	21,23
Wheat	Cas9 nuclease	Protoplasts	21
Sorghum	Cas9 nuclease	Embryos	23
Tobacco	Cas9 nuclease	Protoplasts, leaf tissue	19,20,23
Thale cress	Cas9 nuclease	Protoplasts, seedlings	19,23
Yeast	Cas9 nuclease	*Saccharomyces cerevisiae*	18
Bacteria	Cas9 nuclease	*Streptococcus pneumoniae*, *E. coli*	8
	dCas9 (gene regulation)	*E. coli*	69,70

HEK, human embryonic kidney; iPSCs, induced pluripotent stem cells; UMUC3, human bladder cancer.

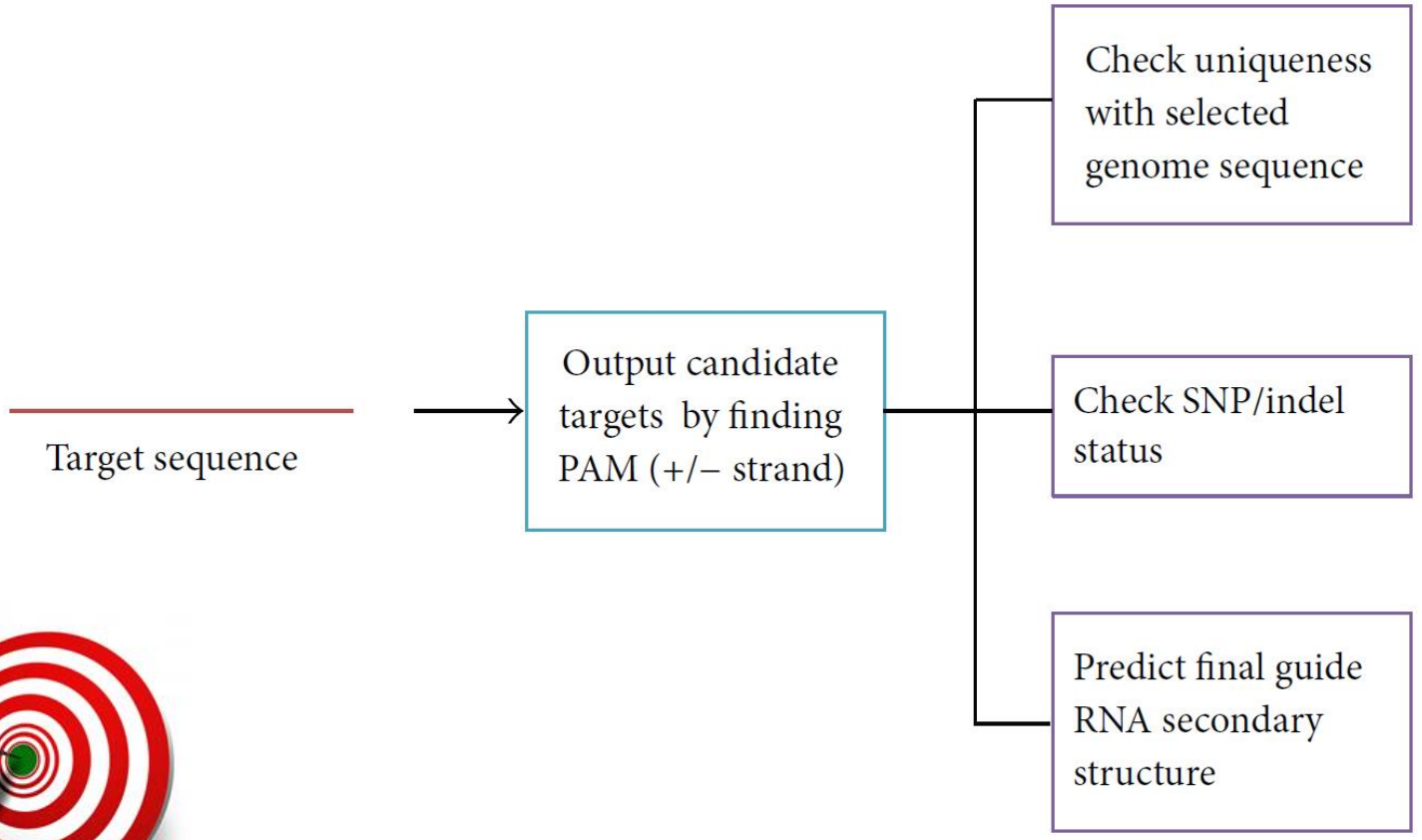
Improving Specificity

gRNA target site design

Double nicking by Cas9 D10A

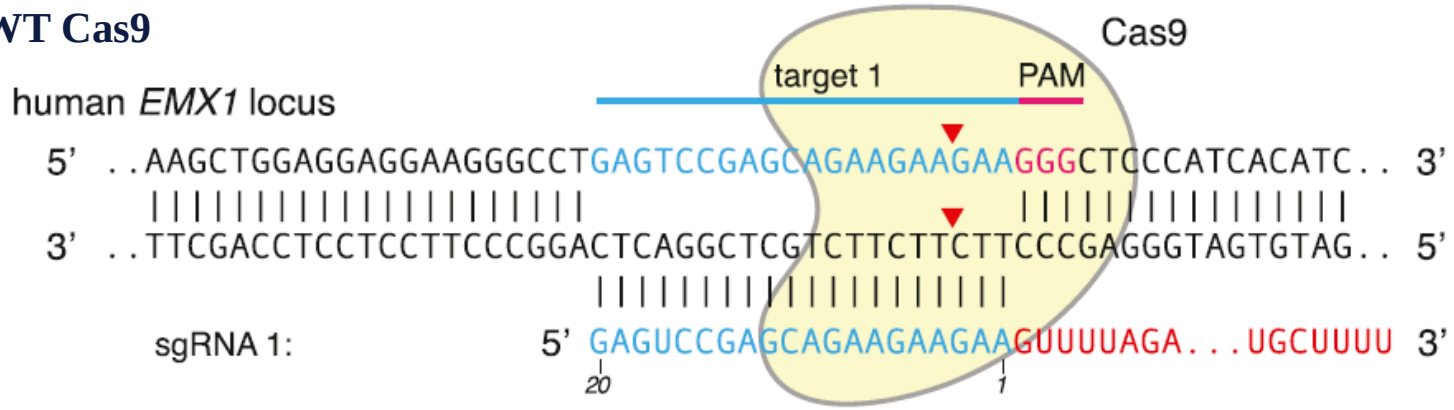
Truncated gRNA

Streamline of guide RNA design platform

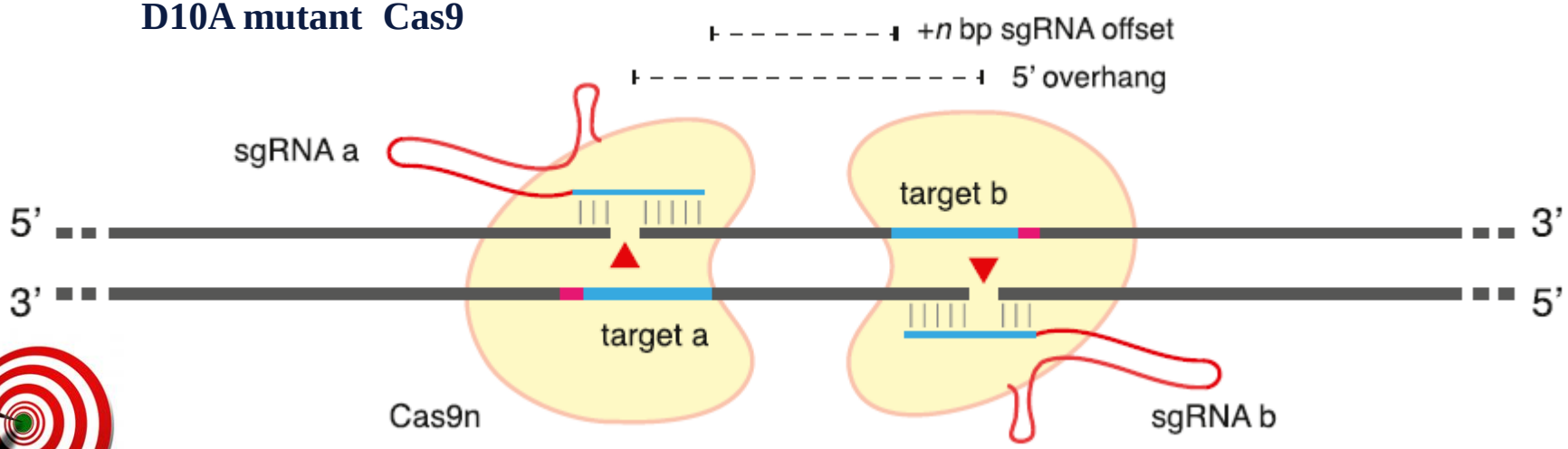


Double Nicking by RNA-Guided CRISPR Cas9

WT Cas9

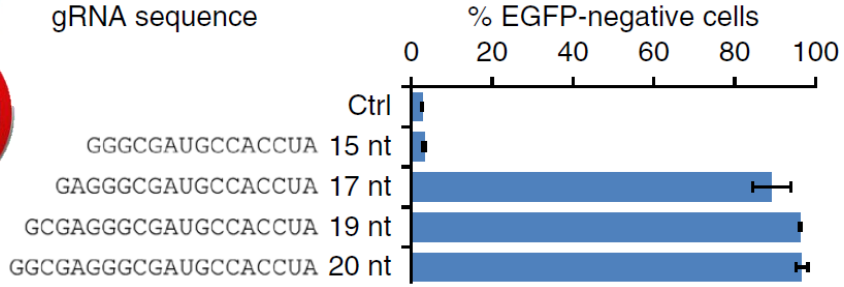


D10A mutant Cas9



(2014)

Improving CRISPR-Cas nuclease specificity using truncated guide RNAs



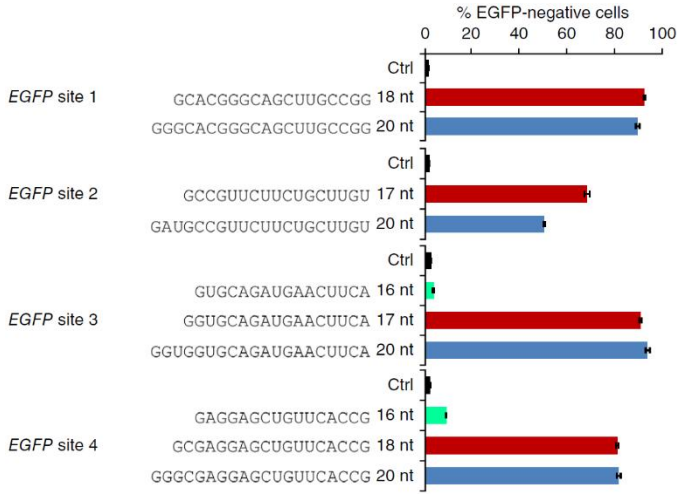


Table 1 Frequencies of indels induced at on- and off-target sites by tru-RGNs and matched standard RGNs in human U2OS.EGFP cells

Target ID	Gene	Full-length target (20 nt)	Indel mutation frequency (%)	Truncated target (17 or 18 nt)	Indel mutation frequency (%)
Target site 1	*VEGFA*	GGGTGGGGGGAGTTTGCTCCtGG	**23.69** (25.68, 21.70)	GTGGGGGGAGTTTGCTCCtGG	**23.93** (28.30, 19.55)
OT1-3	*IGDCC3*	GGATGGAGGGAGTTTGCTCCtGG	**17.25** (20.22, 14.28)	ATGGAGGGAGTTTGCTCCtGG	Not detected
OT1-4	*LOC116437*	GGGAGGGTGGAGTTTGCTCCtGG	**6.23** (6.44, 6.03)	GAGGGTGGAGTTTGCTCCtGG	Not detected
OT1-6	*CACNA2D*	CGGGGGGAGGGAGTTTGCTCCtGG	**3.73** (3.95, 3.50)	GGGGGAGGGAGTTTGCTCCtGG	Not detected
OT1-11		GGGGAAGGGGAAGTTTGCTCCtGG	**10.36** (11.02, 9.69)	GGAAGGGGAAGTTTGCTCCtGG	Not detected
Target site 3	*VEGFA*	GGTGAGTGAGTGTGTGCGTGtGG	**54.08** (55.10, 53.06)	GAGTGAGTGTGTGCGTGtGG	**50.49** (49.24, 51.74)
OT3-1	*(abParts)*	GGTGAGTGAGTGTGTGTGTGaGG	**6.16** (6.71, 5.60)	GAGTGAGTGTGTGTGTGaGG	Not detected
OT3-2	*MAX*	AGTGAGTGAGTGTGTGTGTGgGG	**19.64** (18.58, 20.70)	GAGTGAGTGTGTGTGTGgGG	**5.52** (5.77, 5.27)
OT3-4		GCTGAGTGAGTGTATGCGTGtGG	**7.95** (7.84, 8.06)	GAGTGAGTGTATGCGTGtGG	**1.69** (1.42, 1.95)
OT3-9	*TPCN2*	GGTGAGTGAGTGCGTGCGGtGG	Not Detected	GAGTGAGTGCGTGCGGtGG	Not detected
OT3-17	*SLIT1*	GTTGAGTGAATGTGTGCGTGaGG	**1.85** (1.77, 1.92)	GAGTGAATGTGTGCGTGaGG	Not detected
OT3-18	*COMDA*	TGTGGGTGAGTGTGTGCGTGaGG	**6.16** (6.72, 5.60)	GGGTGAGTGTGTGCGTGaGG	Not detected
OT3-20		AGAGAGTGAGTGTGTGCATGaGG	**10.47** (9.39, 11.55)	GAGTGAGTGTGTGCATGaGG	Not detected
Target site 4	*EMX1*	GAGTCCGAGCAGAAGAAGAAgGG	**41.56** (41.76, 41.37)	GTCCGAGCAGAAGAAGAAgGG	**43.01** (43.89, 42.15)
OT4-1	*HCN1*	GAGTTAGAGCAGAAGAAGAAaGG	**19.26** (18.54, 19.99)	GTTAGAGCAGAAGAAGAAaGG	Not detected
OT4_53[a]	*MFAP1*	GAGTCTAAGCAGAAGAAGAAgAG	**4.37** (3.79, 4.96)	GTCTAAGCAGAAGAAGAAgAG	Not detected

Mutation frequencies were assessed by T7EI assay with means (bold text) of duplicate measurements (in brackets) shown. OT, off-target site.

[a]Off-target site OT4_53 is the same as *EMX1* target 3 OT31 from ref. 5.

Off-target Analysis

Off-target sites analysis

Mutation frequencies at on-target and potential off-target sites

Cho et al., *Genome Res.* 2013, doi:10.1101/gr.162339.113

SEOUL NATIONAL UNIVERSITY

RGEN Tools
(RNA-guided engineered nucleases)

GENES

Cas-OFFinder (potential off-target finder)	Microhomology-associated Score Calculator

http://www.rgenome.net/cas-offinder/

Cas-OFFinder

Citation info: Bioinformatics (2014) 30 (10): 1473-1475, doi:10.1093/bioinformatics/btu048

Submit a new searching job, or download an off-line version of Cas-OFFinder here.

Job title (Optional):

E-mail (Optional, **but strongly recommended !!**):

* Searching job is working in sequence for many input data, therefore it would be convenient to receive the results by e-mail.

PAM Type

CRISPR/Cas-derived RNA-guided Endonucleases (RGENs)

- (•) SpCas9 from Streptococcus pyogenes: 5'-NGG-3'
- ( ) SpCas9 from Streptococcus pyogenes: 5'-NRG-3' (R = A or G)
- ( ) StCas9 from Streptococcus thermophilus: 5'-NNAGAAW-3' (W = A or T)
- ( ) NmCas9 from Neisseria meningitidis: 5'-NNNNGMTT-3' (M = A or C)

Target Genome

Genomes

- (•) Homo sapiens (GRCh38/hg38) - Human (02 April 2014 Updated)
- ( ) Homo sapiens (hg19) - Human
- ( ) Mus musculus (mm10) - Mouse
- ( ) Bos taurus (bosTau7) - Cow
- ( ) Canis familiaris (canFam3) - Dog
- ( ) Rattus norvegicus (rn5) - Rat
- ( ) Sus scrofa (susScr3) - Pig
- ( ) Danio rerio (danRer7) - Zebrafish
- ( ) Drosophila melanogaster (dm3) - Fruit fly
- ( ) Caenorhabditis elegans (ce10) - C. elegans
- ( ) Arabidopsis thaliana (TAIR10) - Thale cress
- ( ) Oryza sativa (OSv4) - Rice
- ( ) Solanum lycopersicum (SL2.4) - Tomato
- ( ) Zea mays (AGPv3) - Corn
- ( ) Macaca mulatta(rheMac3) - Monkey

Query Sequences

Query sequences (5' to 3'), one sequence per line.
Please write crRNA sequences **without PAM sequences**.
The length of each query sequence should be **20** bp long!

Mismatch Number (less than) 0

```
Example (for SpCas9):
AAAAAGGGGGVVVVVWWWWW
AAAAATTTTGGGGGRRRRR
...
```

Please note that the length of query sequences should be 20bp for SpCas9, 18bp for StCas9, and 24bp for NmCas9.

Mixed bases are allowed.

Submit

RGEN Tools
(RNA-guided engineered nucleases)

SEOUL NATIONAL UNIVERSITY

Cas-OFFinder (potential off-target finder)	Microhomology-associated Score Calculator

Each result will be kept on server for 3 days only.

URL of this page: http://www.rgenome.net/cas-offinder/result?hash=aa13ff689b9f3d90fe54b17be39041d8

Job ID	Title	Submit Date	Status
2843	test	May 27, 2014, 5:31 p.m.	Finished! Download result

Output file

- First column - given query sequence
- Second column - chromosome title
- Third column - position of the potential off-target site
- Forth column - actual sequence located at the position (mismatched bases noted in lowercase letters)
- Fifth column - indicates forward strand(+) or reverse strand(-) of the found sequence
- Last column - the number of the mismatched bases ('N' in PAM sequence are not counted as mismatched bases)

An example output file:

```
GGCCGACCTGTCGCTGACGCNNN chr8 49679     GGgCatCCTGTCGCaGACaCAGG + 5
GGCCGACCTGTCGCTGACGCNNN chr8 517739    GcCCtgCaTGTgGCTGACGCAGG + 5
GGCCGACCTGTCGCTGACGCNNN chr8 599935    tGCCGtCtTcTCcCTGACGCCAG - 5
GGCCGACCTGTCGCTGACGCNNN chr8 5308348   GGCaGgCCTGgCttTGACGCAGG - 5
GGCCGACCTGTCGCTGACGCNNN chr8 9525579   GGCCcAgCTGTtGCTGAtGaAAG + 5
GGCCGACCTGTCGCTGACGCNNN chr8 12657177  GGCCcACCTGTgGCTGcCcaTAG - 5
GGCCGACCTGTCGCTGACGCNNN chr8 12808911  GGCCGACCaGgtGCTccCGCCGG + 5
GGCCGACCTGTCGCTGACGCNNN chr8 21351922  GGCCcACCTGaCtCTGAgGaCAG - 5
GGCCGACCTGTCGCTGACGCNNN chr8 21965064  GGCCGtCCTGcgGCTGctGCAGG - 5
GGCCGACCTGTCGCTGACGCNNN chr8 22409058  GcCCGACCccTCcCcGACGCCAG + 5
...
```

Our Service

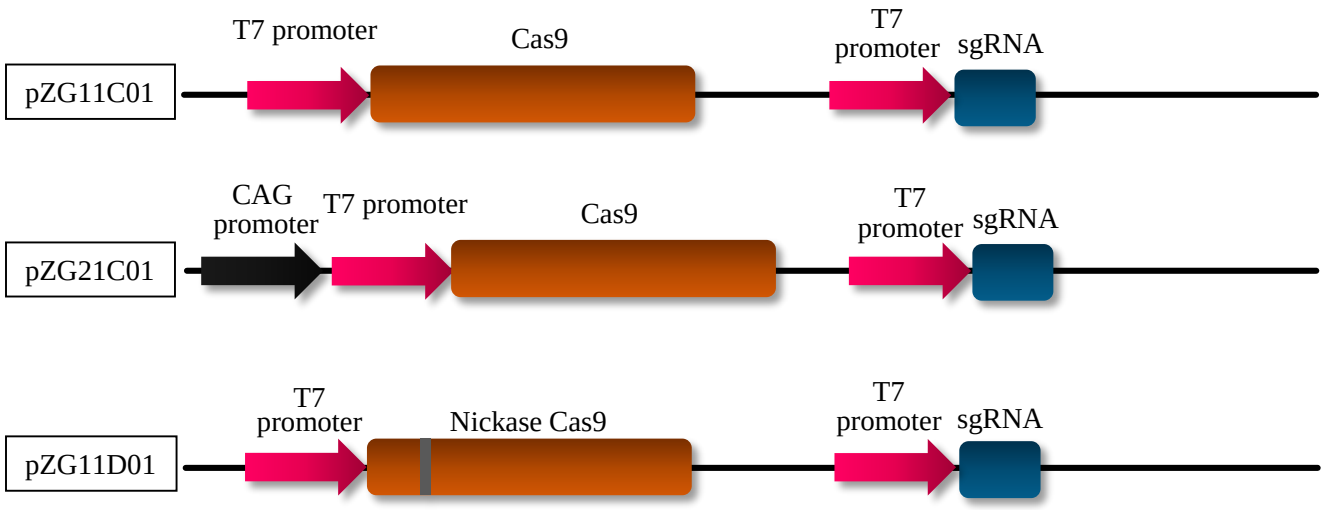
Zgene Biotech CRISPR System

The most comprehensive service

- For most model species
- Meet the needs of antibiotics or fluorescents selection
- Technical support and peripheral products

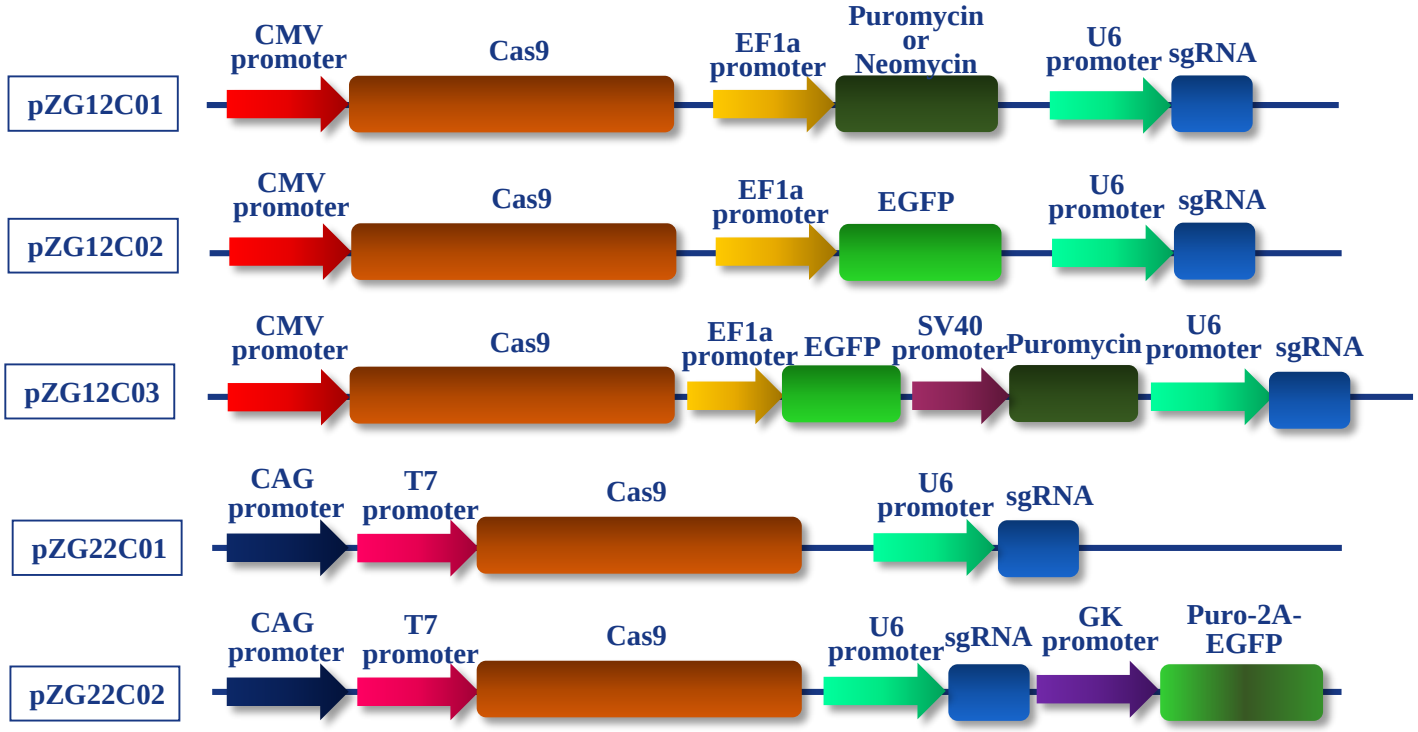
CRISPR-Cas9 Genome Editing System

For In-vitro Transcription CRISPR/Cas9 Vectors



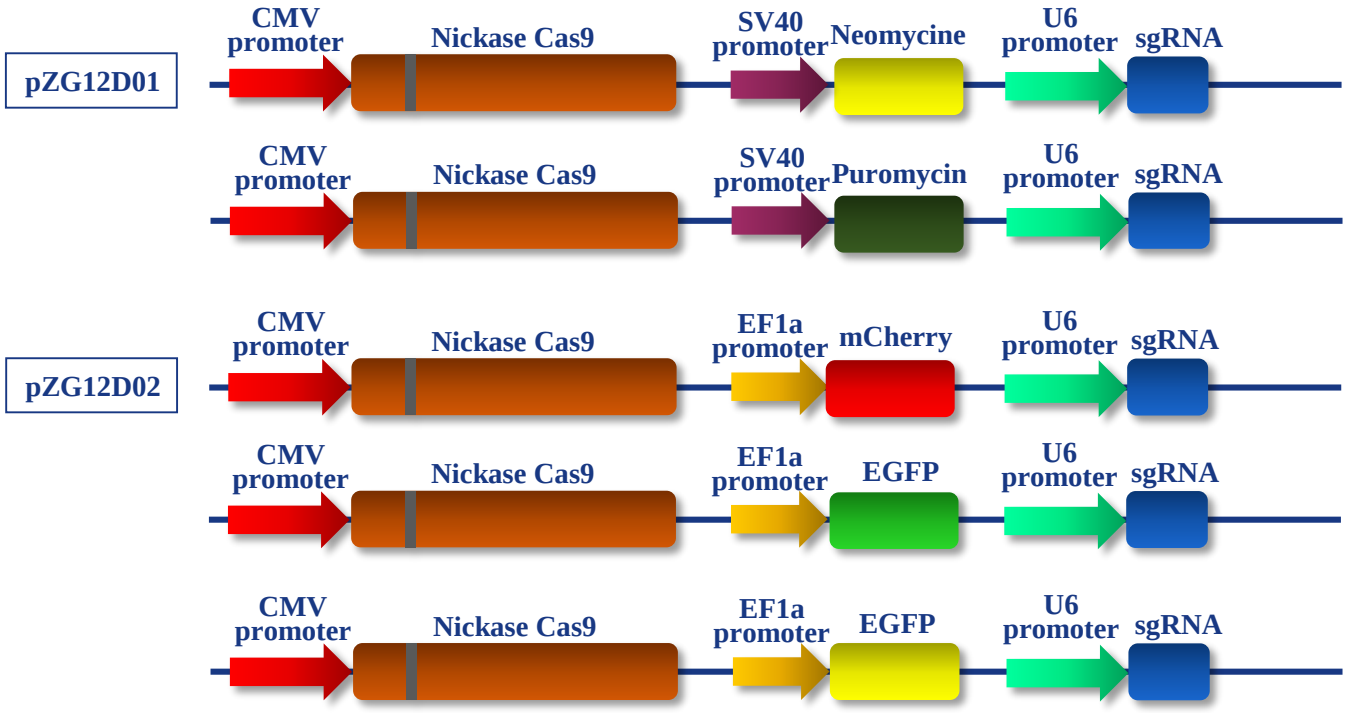
CRISPR-Cas9 Genome Editing System

For Cell Line CRISPR/Cas9 Vectors



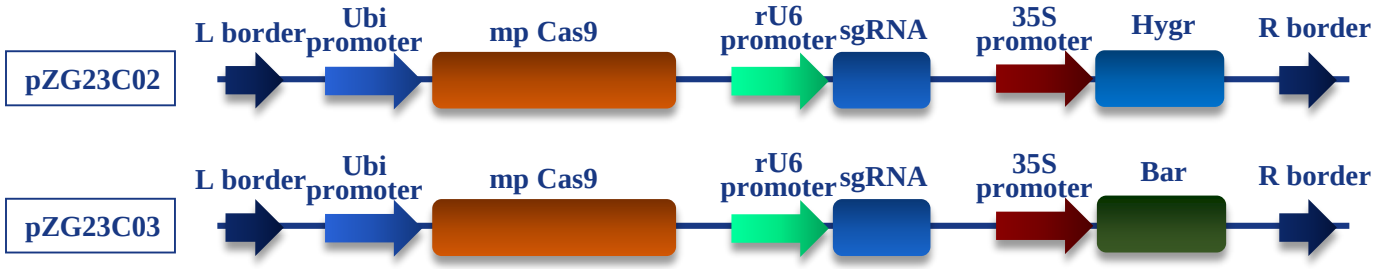
CRISPR-Cas9 Genome Editing System

For Cell Line CRISPR/Nickase Cas9 Vectors

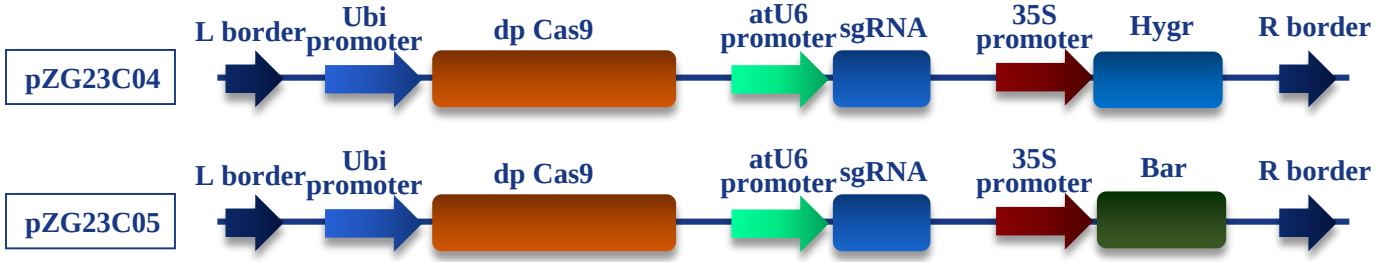


CRISPR-Cas9 Genome Editing System

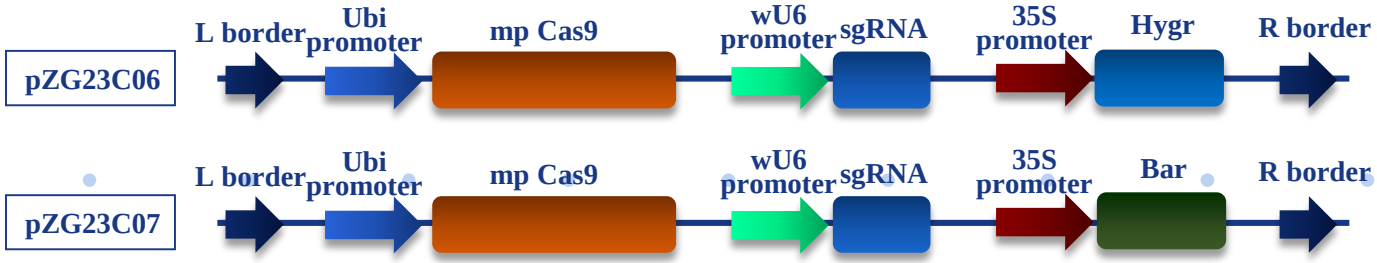
For Rice Plants CRISPR/Cas9Vectors



For Dicots Plants CRISPR/Cas9 Vectors



For Monocot Plants CRISPR/Cas9 Vectors



CRISPR & TALEM Service

3 Days

Target gene information → Target sites design

~15 Working Days

Vector construction → Confirm the selection

Starting your research → After-sales service

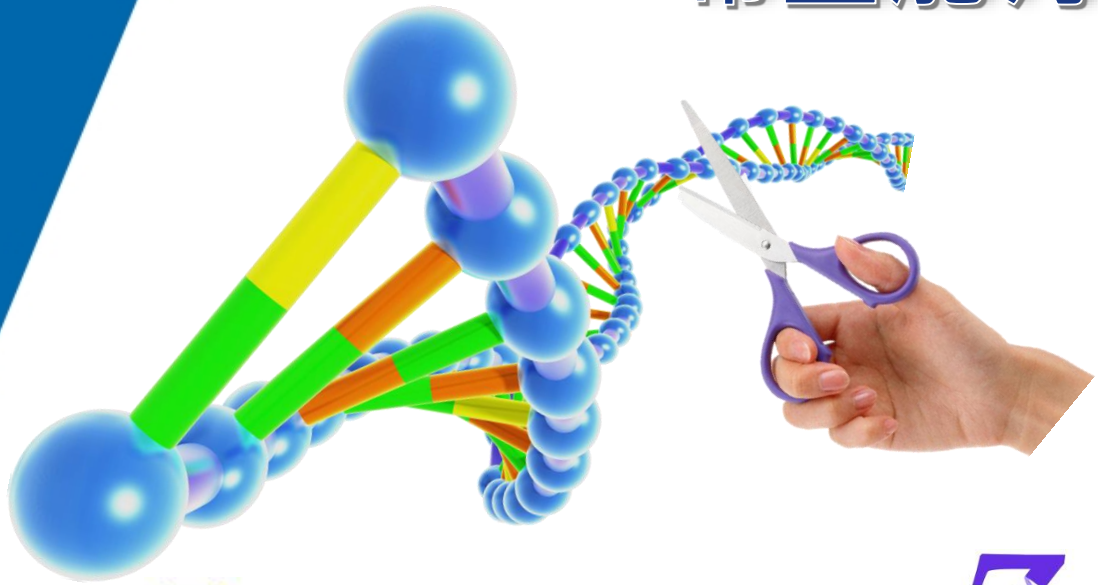
Client

ZGene

力鈞為全球頂尖研究單位提供服務

力鈞生物科技

希望能有機會為您服務



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