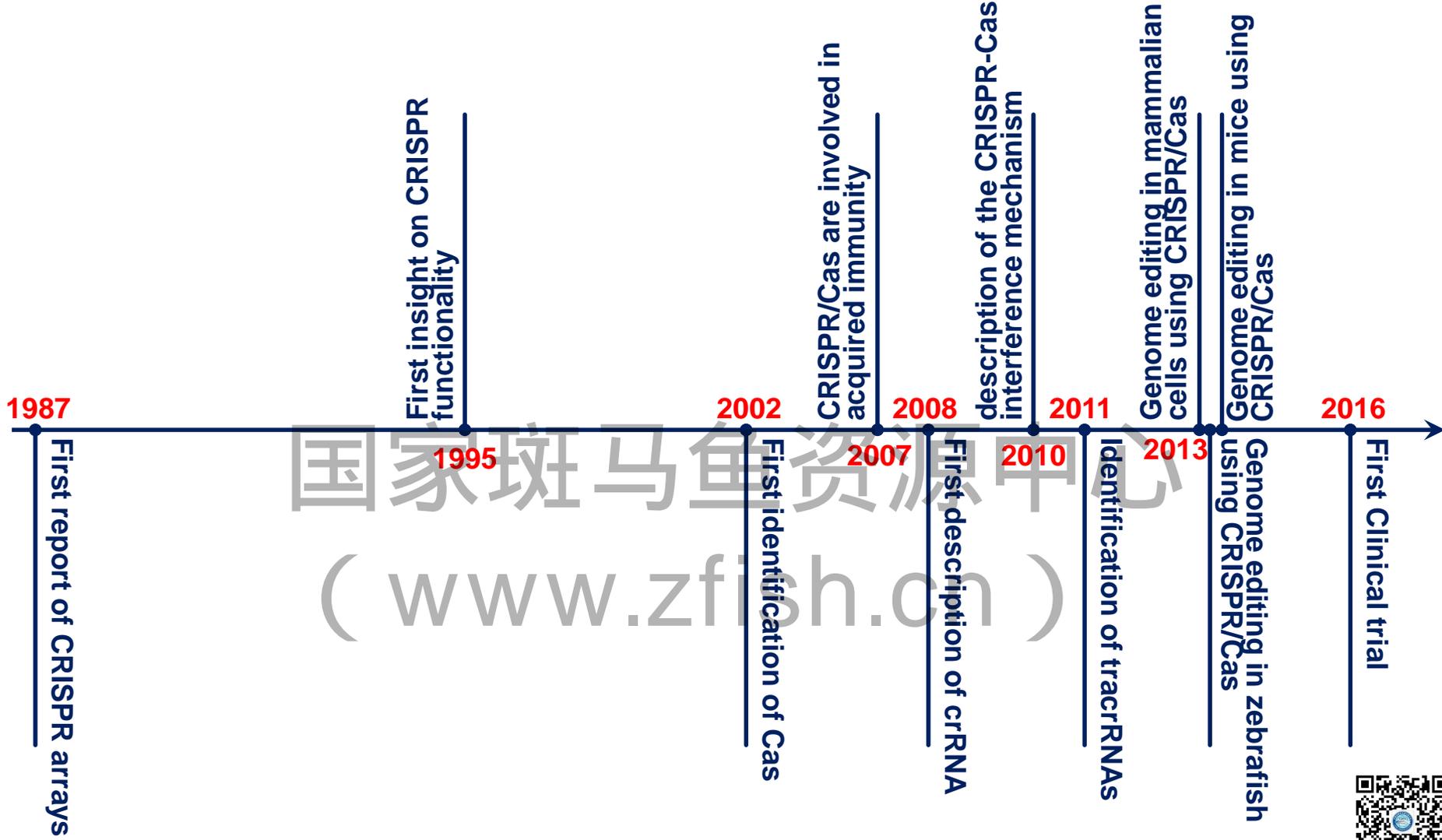


Applications of CRISPR/Cas System

国家斑马鱼资源中心
潘鲁媛
第八期全国技术培训
(www.zfish.cn)
2018年4月



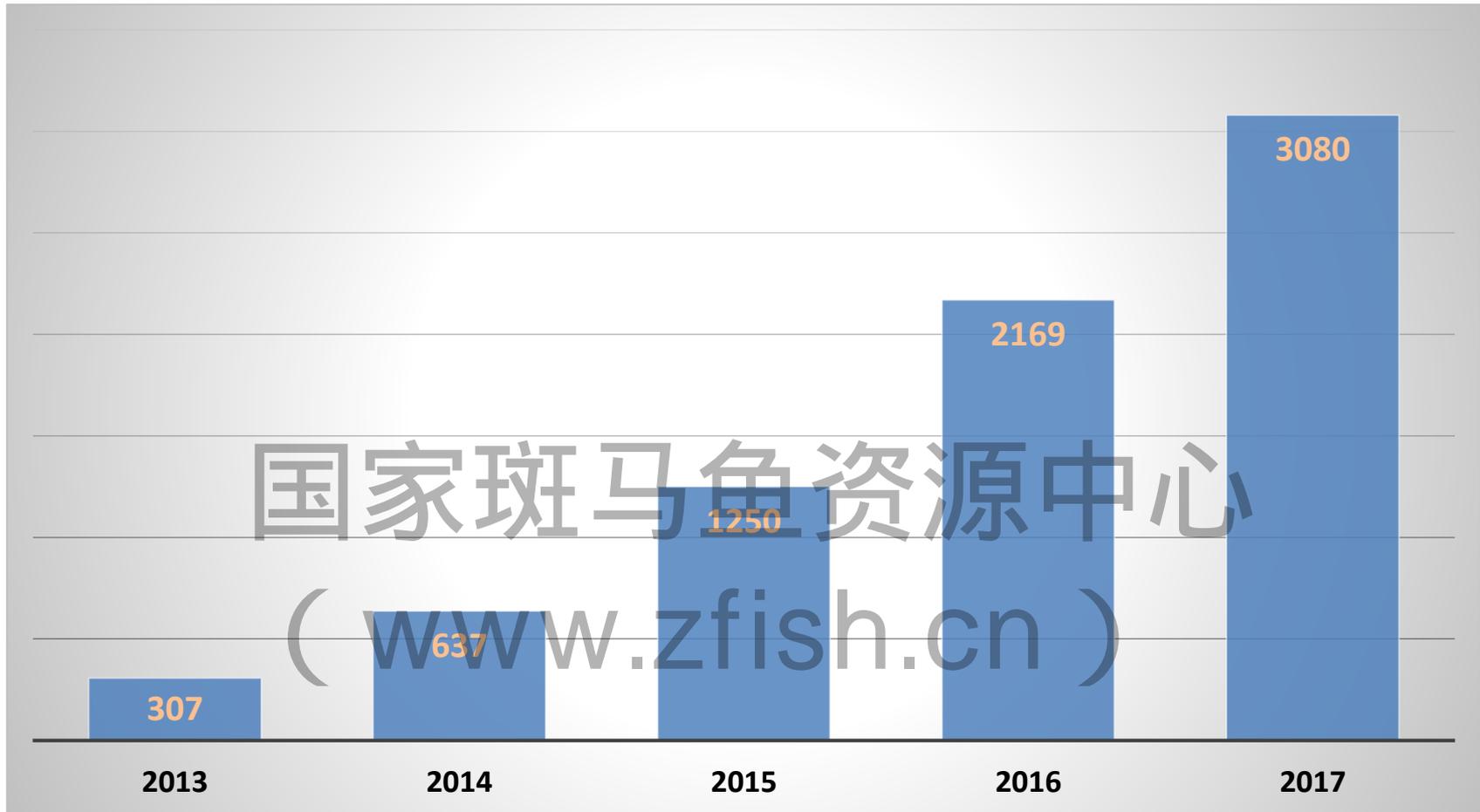
the History of CRISPR/Cas



国家斑马鱼资源中心
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Explosive Growth of CRISPR/Cas-related research

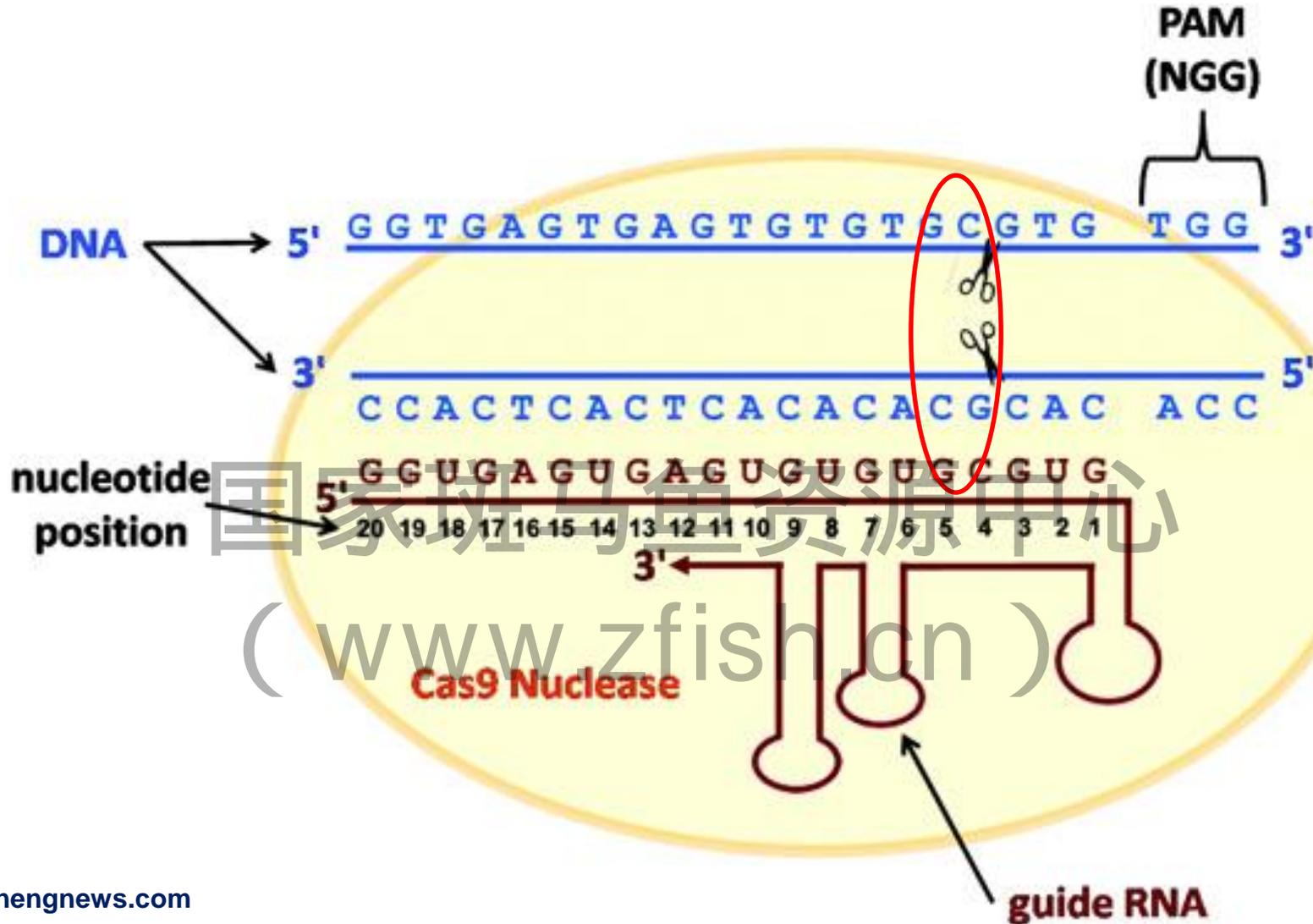


Applications of CRISPR/Cas System

- In plant engineering
- In preclinical/clinical application
- In drug target identification
- In modulating gene expression
- single-base substitutions
- In zebrafish

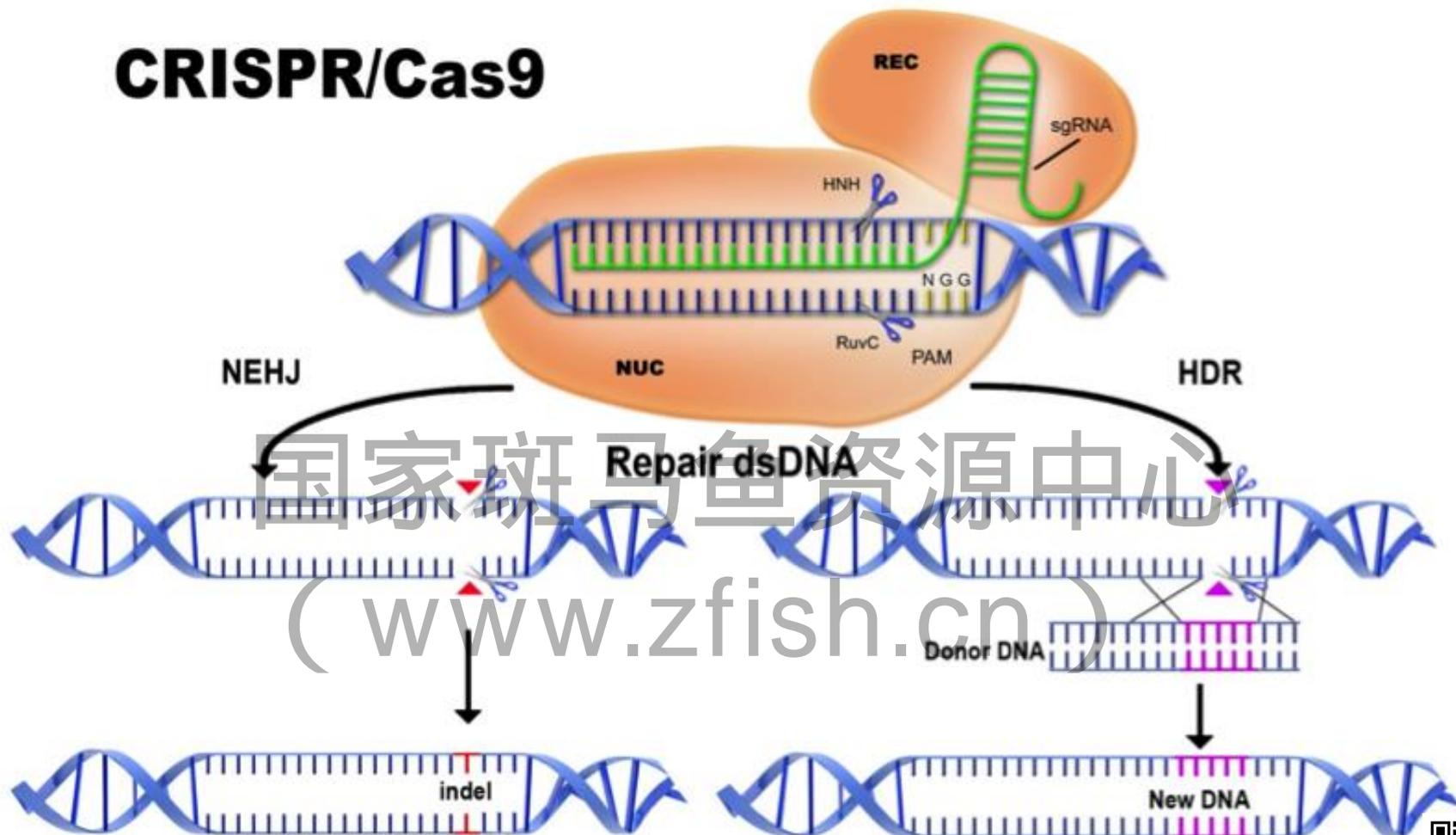


Genome-editing mechanism of CRISPR/Cas



Genome-editing mechanism of CRISPR/Cas

CRISPR/Cas9



Nouns

- SpCas9: most commonly used, from *Streptococcus pyogenes*
- Cpf1: CRISPR-associated endonuclease in *Prevotella* and *Francisella* 1, crRNA, 5' TTTN PAM, cut staggered end
- HFCas9: high-fidelity Cas9 (N497A, R661A, Q695A, and Q926A)
- nCas9: nickase cas9
- dCas9: dead cas9
- CRISPRi vs CRISPRa: interference and activation



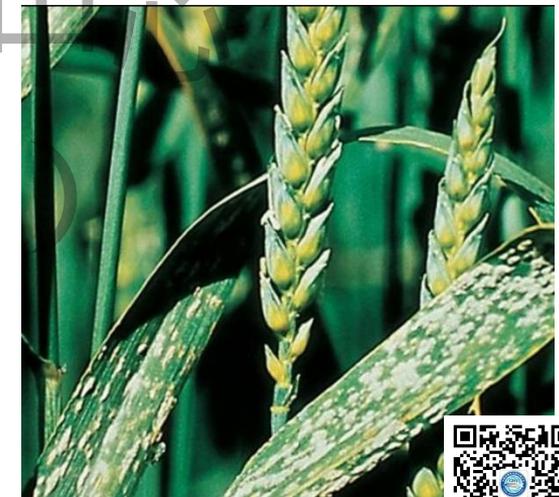
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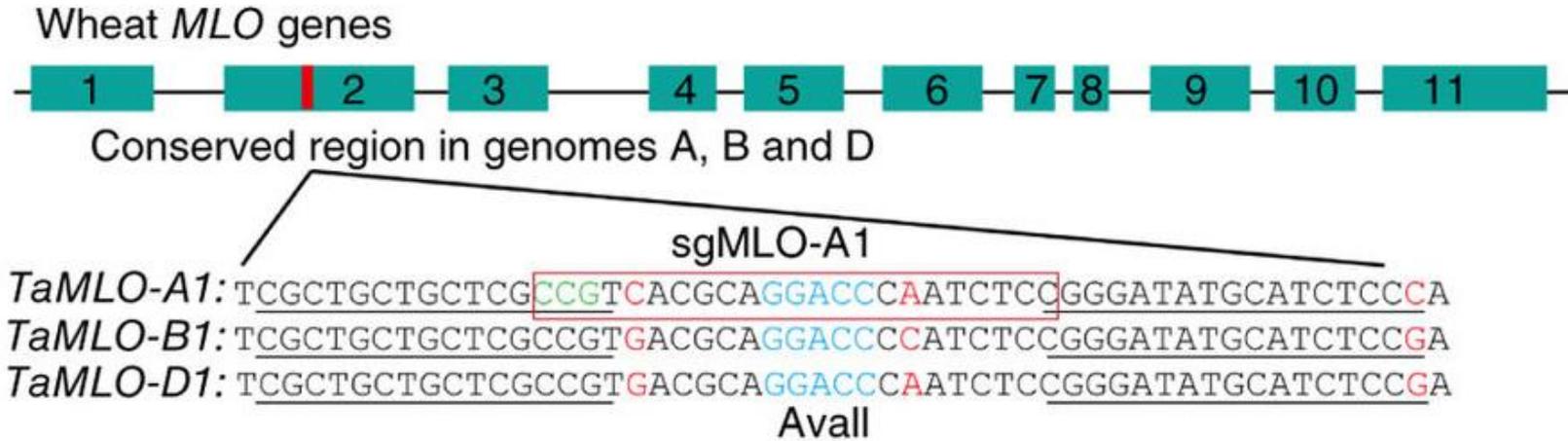


In Plants: deal with polyploidy

- Bread wheat : ~20% of all calories consumed by humans.
- Bread wheat is an allohexaploid ($2n = 42$, AABBDD).
- Powdery mildew is one of the most destructive plant pathogens worldwide.
- MLO (MILDEW-RESISTANCE LOCUS) a negative regulator in PM-resistance.
- 3 MLO homoeologs in bread wheat (TaMLO-A1, TaMLO-B1 and TaMLO-D1) are 98% and 99% identical.



In Plants: deal with polyploidy



国家斑马鱼资源中心
 WT tamlo-aa tamlo-bb tamlo-dd tamlo-aabb tamlo-aadd tamlo-aabbdd



Applications of CRISPR/Cas System

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Clinical Application: gene therapy

- Gene therapy: infectious diseases, PID(primary immunodeficiency), hemoglobinopathies, leukemia, hemophilia, metabolic disorders, muscular dystrophy, cancer
- Delivery vehicles: AAV (Adeno-associated virus) vectors, LNPs (Lipid nanoparticles), electroporation
- Cytotoxicity: specificity, target, dosage



Gene therapy: Muscular dystrophy

Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

Chengzu Long,^{1,2,3*} Leonela Amoasii,^{1,2,3*} Alex A. Mireault,^{1,2,3} John R. McAnally,^{1,2,3} Hui Li,^{1,2,3} Efrain Sanchez-Ortiz,^{1,2,3} Samadrita Bhattacharyya,^{1,2,3} John M. Shelton,⁴ Rhonda Bassel-Duby,^{1,2,3} Eric N. Olson^{1,2,3,†}

Science. 2016;
351(6271):400-403.
UT Southwestern
Medical Center

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,^{1,2} Chady H. Hakim,³ David G. Ousterout,^{1,2} Pratiksha I. Thakore,^{1,2} Eirik A. Moreb,^{1,2} Ruth M. Castellanos Rivera,⁴ Sarina Madhavan,^{1,2} Xiufang Pan,³ F. Ann Ran,^{5,6} Winston X. Yan,^{5,7,8} Aravind Asokan,⁴ Feng Zhang,^{5,9,10,11} Dongsheng Duan,^{3,12} Charles A. Gersbach^{1,2,13*}

Science. 2016;
351(6271):403-407.
DukeU

In vivo gene editing in dystrophic mouse muscle and muscle stem cells

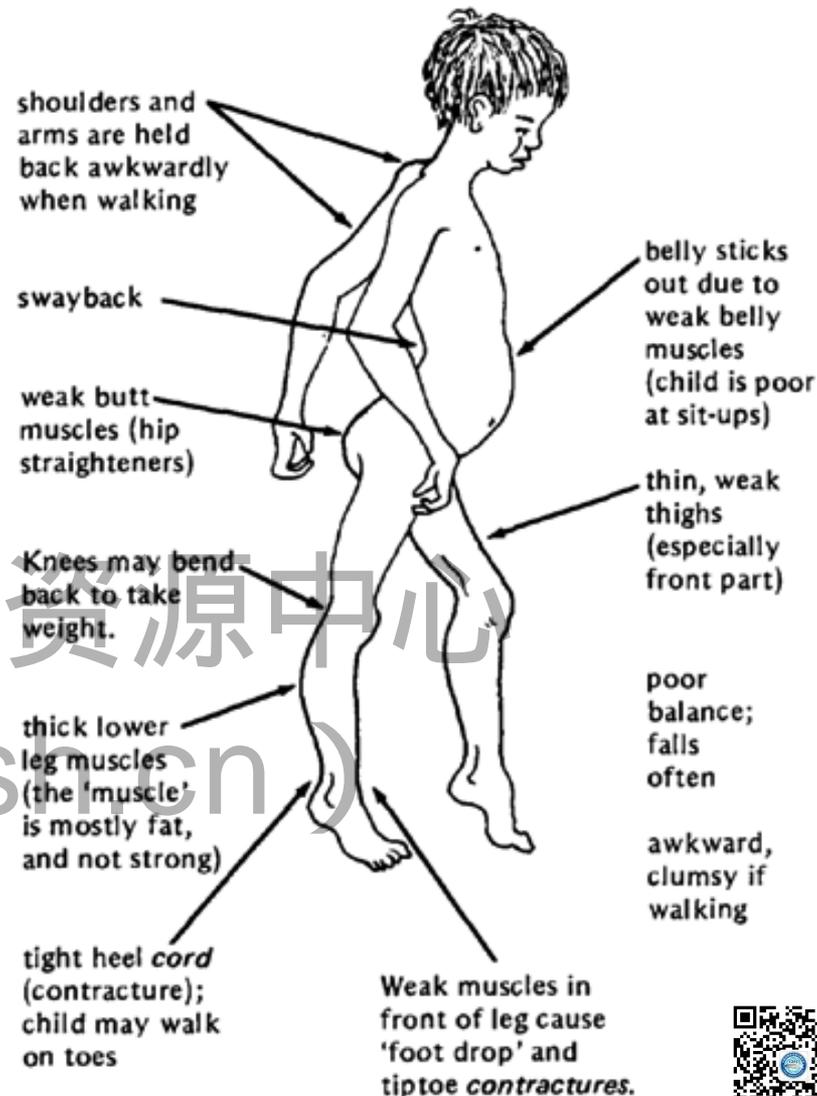
Mohammadsharif Tabebordbar,^{1,2*} Kexian Zhu,^{1,3*} Jason K. W. Cheng,¹ Wei Leong Chew,^{2,4} Jeffrey J. Widrick,⁵ Winston X. Yan,^{6,7} Claire Maesner,¹ Elizabeth Y. Wu,^{1†} Ru Xiao,⁸ F. Ann Ran,^{6,7} Le Cong,^{6,7} Feng Zhang,^{6,7} Luk H. Vandenberghe,⁸ George M. Church,⁴ Amy J. Wagers^{1‡}

Science. 2016;
351(6271):407-411.
Harvard Medical



Duchenne muscular dystrophy

- DMD: X-linked
- 1 in 5,000 males at birth, most common
- Muscle loss, Scoliosis, intellectual disability
- dystrophin: maintain the muscle fiber's cell membrane
- No known cure

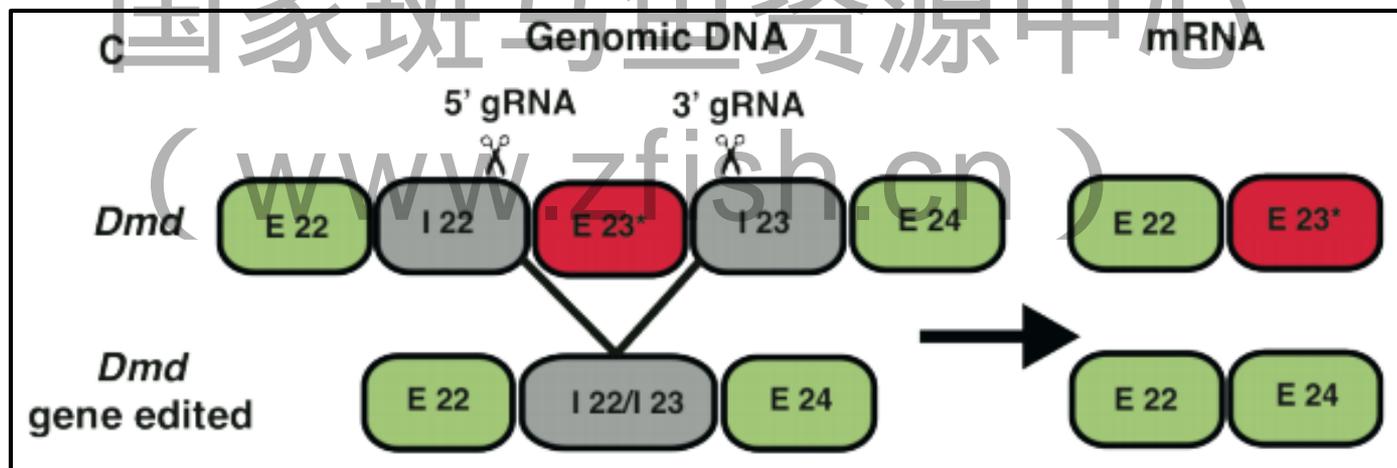
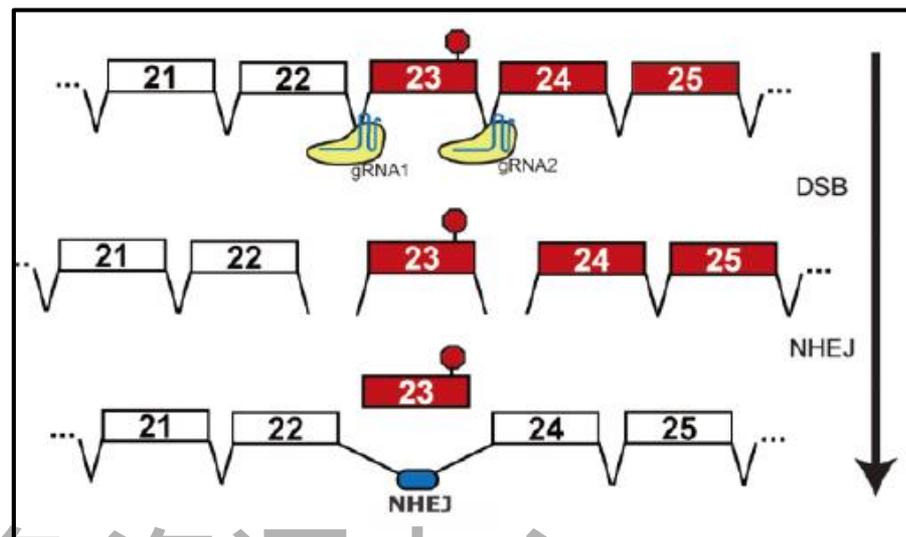
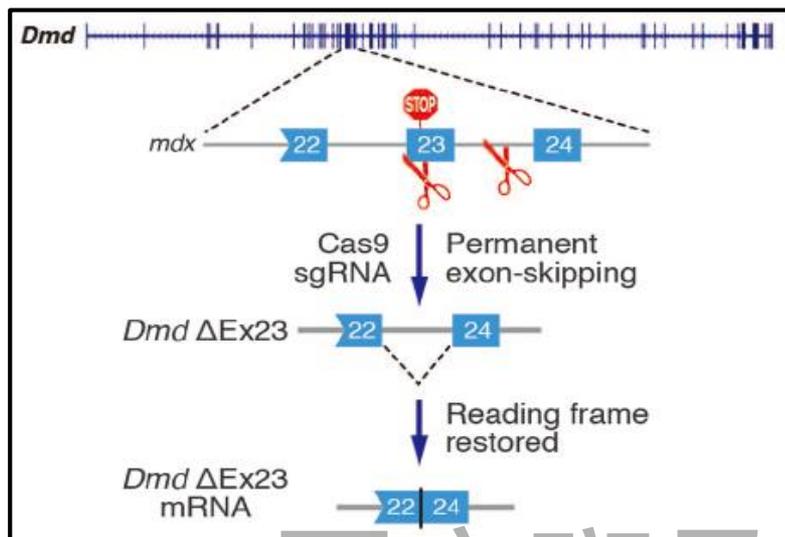


Gene therapy: Muscular dystrophy

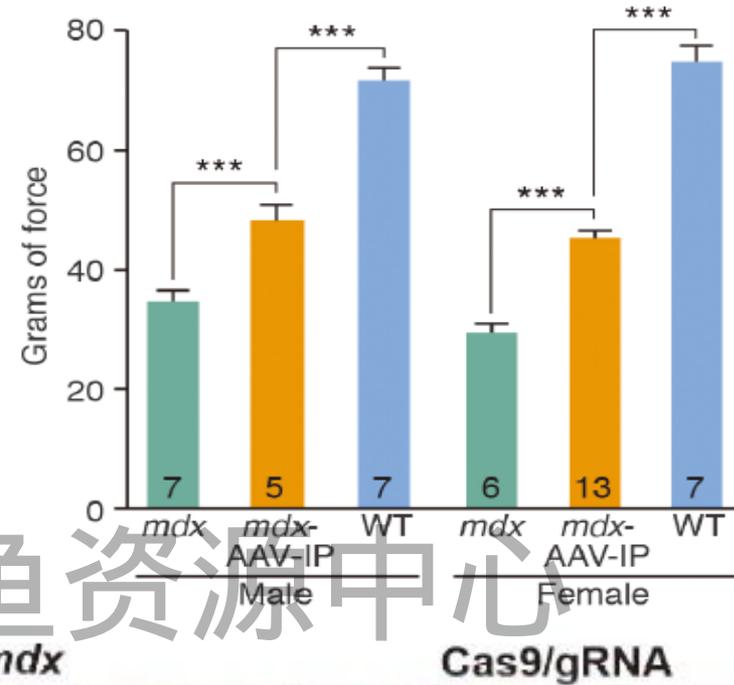
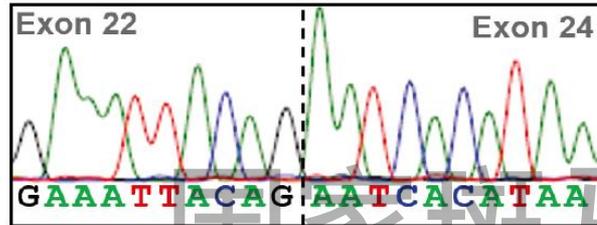
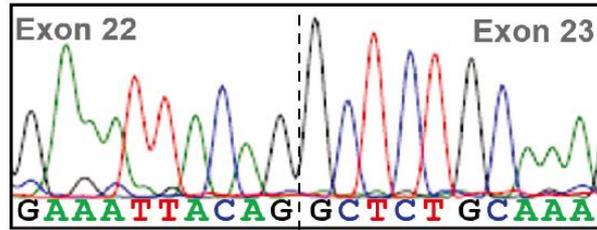
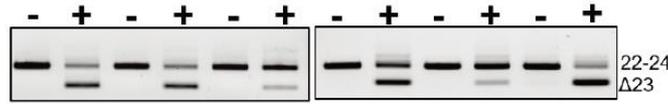
	400	403	407
Disease	DMD	DMD	DMD
Gene	dystrophin	dystrophin	dystrophin
Target	exon23	exon23	exon23
strategy	exon skip	exon skip	exon skip
Delivery	AAV	AAV	AAV
tissue	cardiac and skeletal muscle	myofibers and cardiac muscle	myofibers, cardiomyocytes, and muscle stem cells
mice age	P1, P12, P18	P2, 6week,	P3



Gene therapy: Muscular dystrophy



Gene therapy: Muscular dystrophy

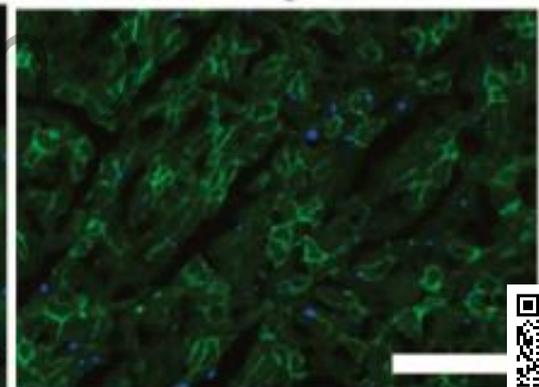
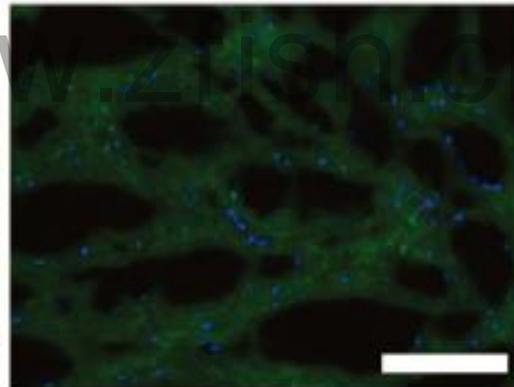
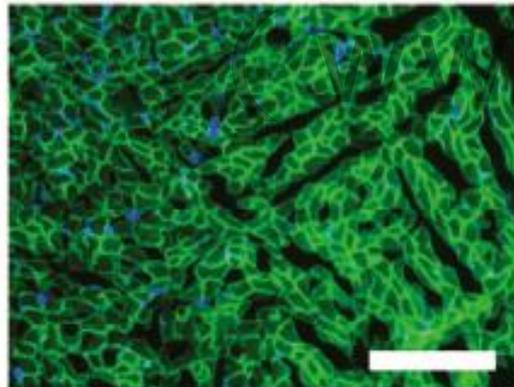


D

WT

mdx

Cas9/gRNA



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Genome-editing and drug-target

- Target identification via library screens
- Identification of critical domains within targets
- Studies of drug-target interactions
- Linking disease-associated SNPs to targets
- validation of human cellular disease models



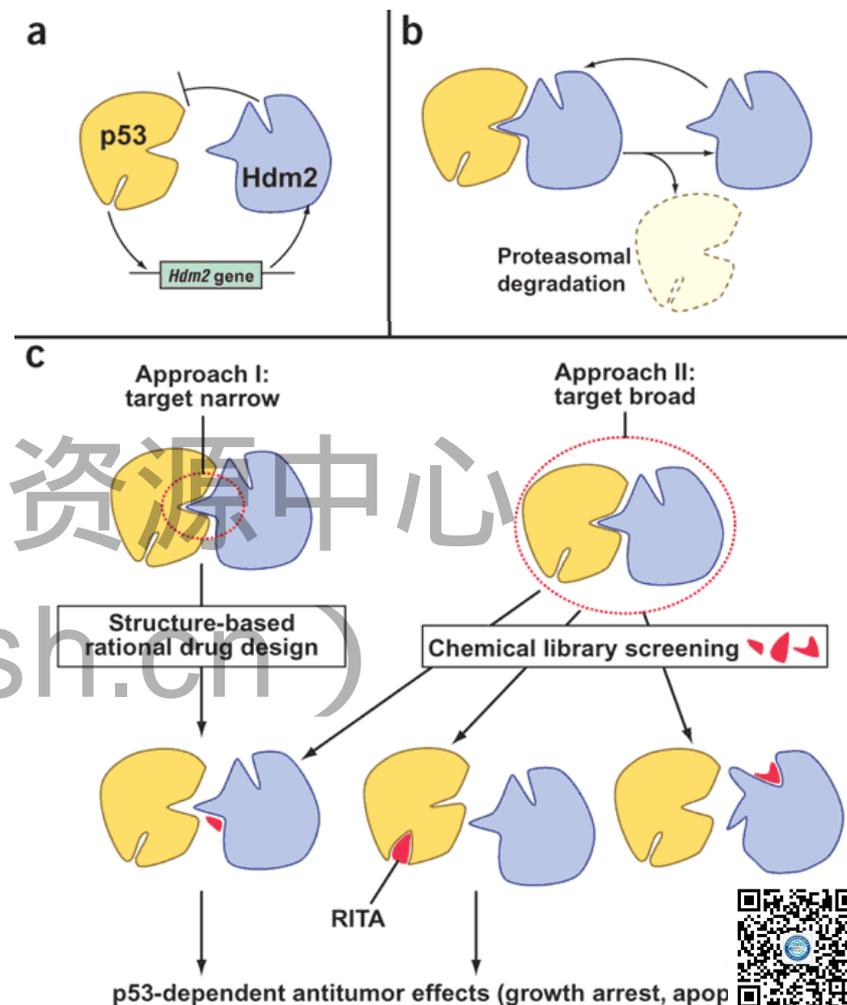
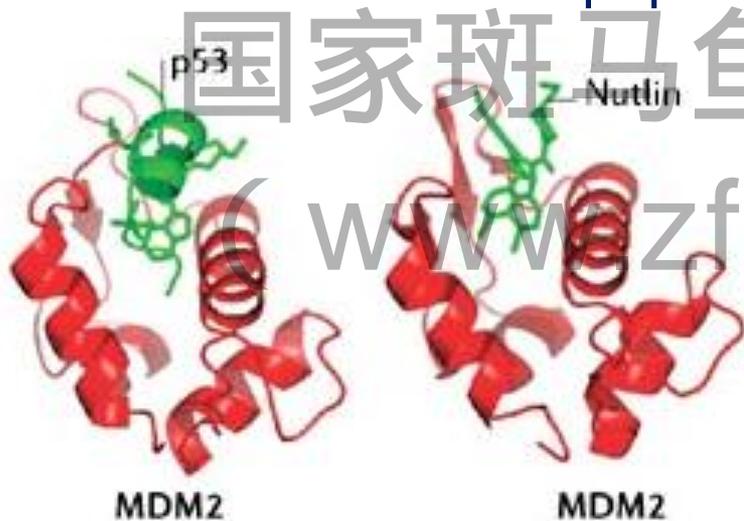
Genome-editing and drug-target

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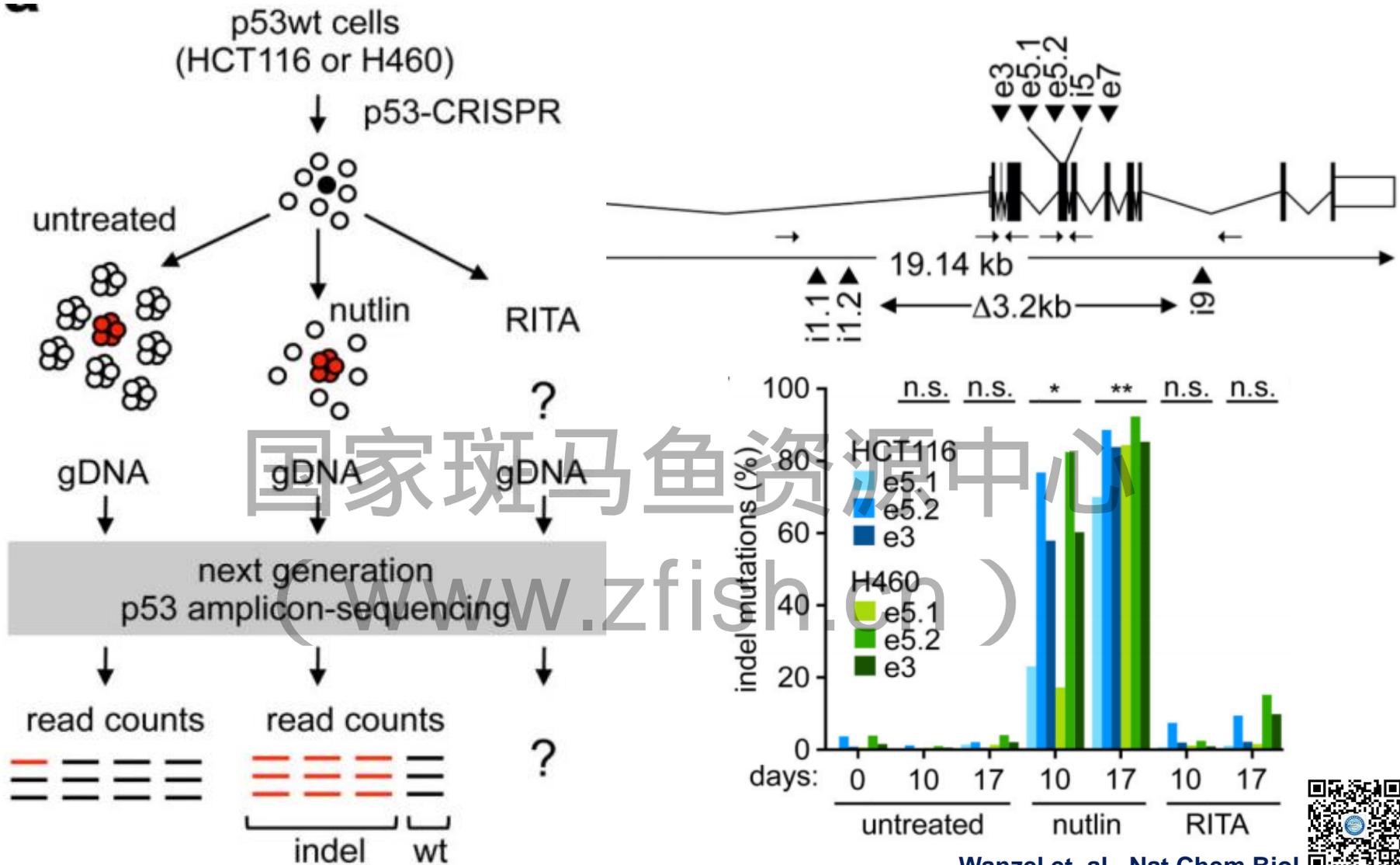


Target validation for p53-reactivating in lung and colorectal cancer

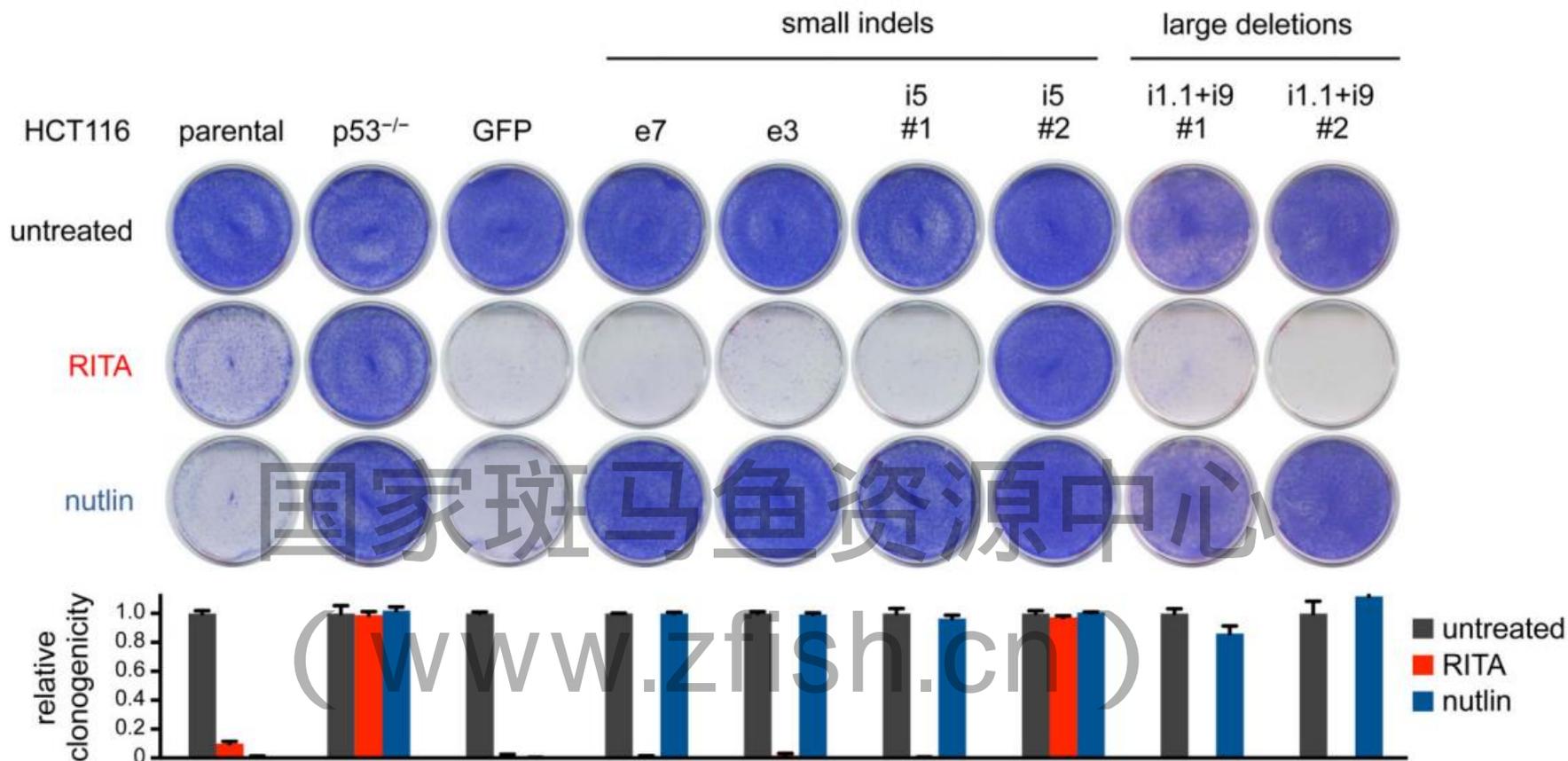
- ~ half of all cancer patients have a mutated TP53 gene
- p53's activity is inhibited by Mdm2
- nutlin: inhibitors of p53-Mdm2 interaction, release p53
- RITA: reactivation of p53 and induction of tumor cell apoptosis



Nutlin vs RITA



Nutlin vs RITA



Nutlin: WT p53-dependent

RITA: WT p53-independent

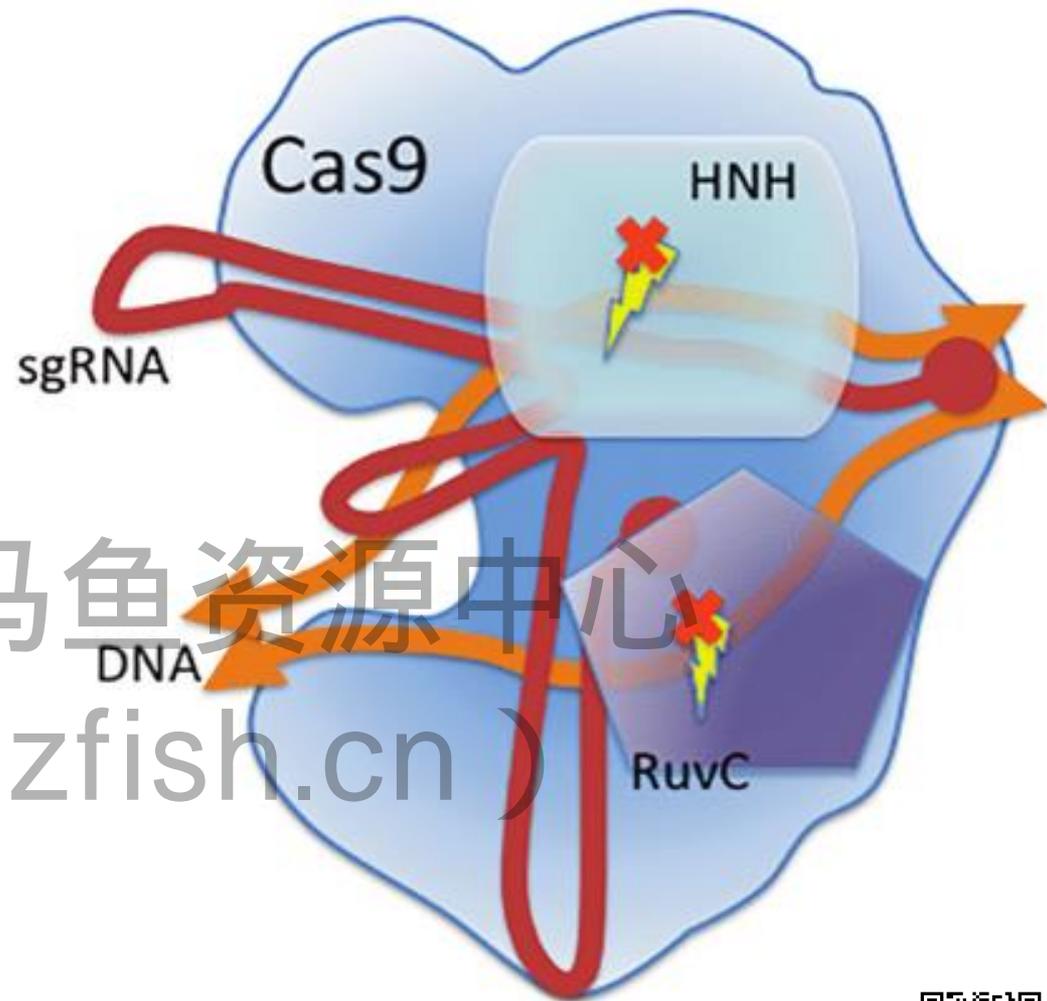
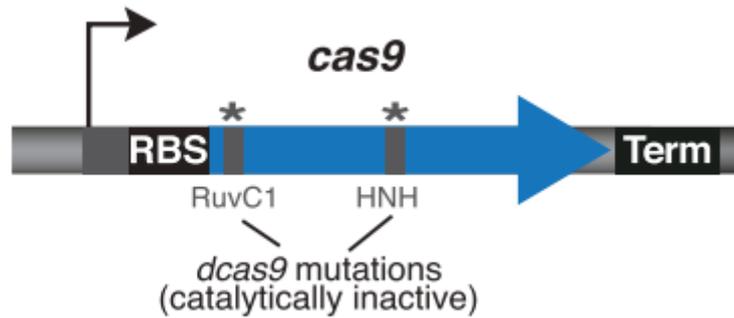


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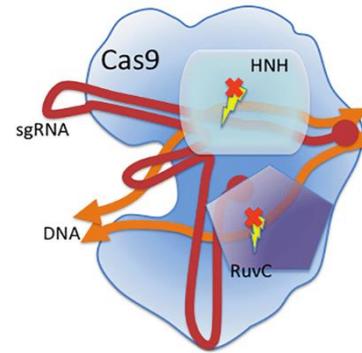
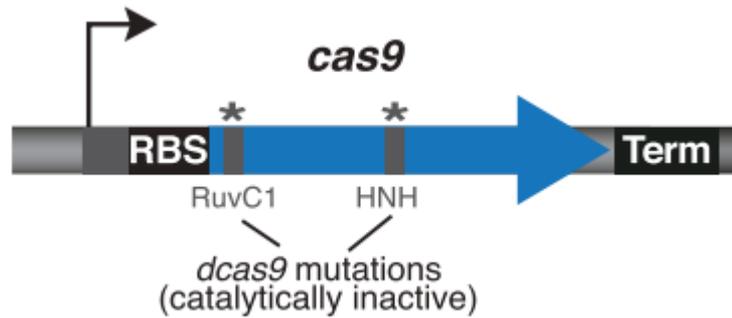
Modulating Gene Expression: dCas9 and CRISPRi



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Modulating Gene Expression: dCas9 and CRISPRi

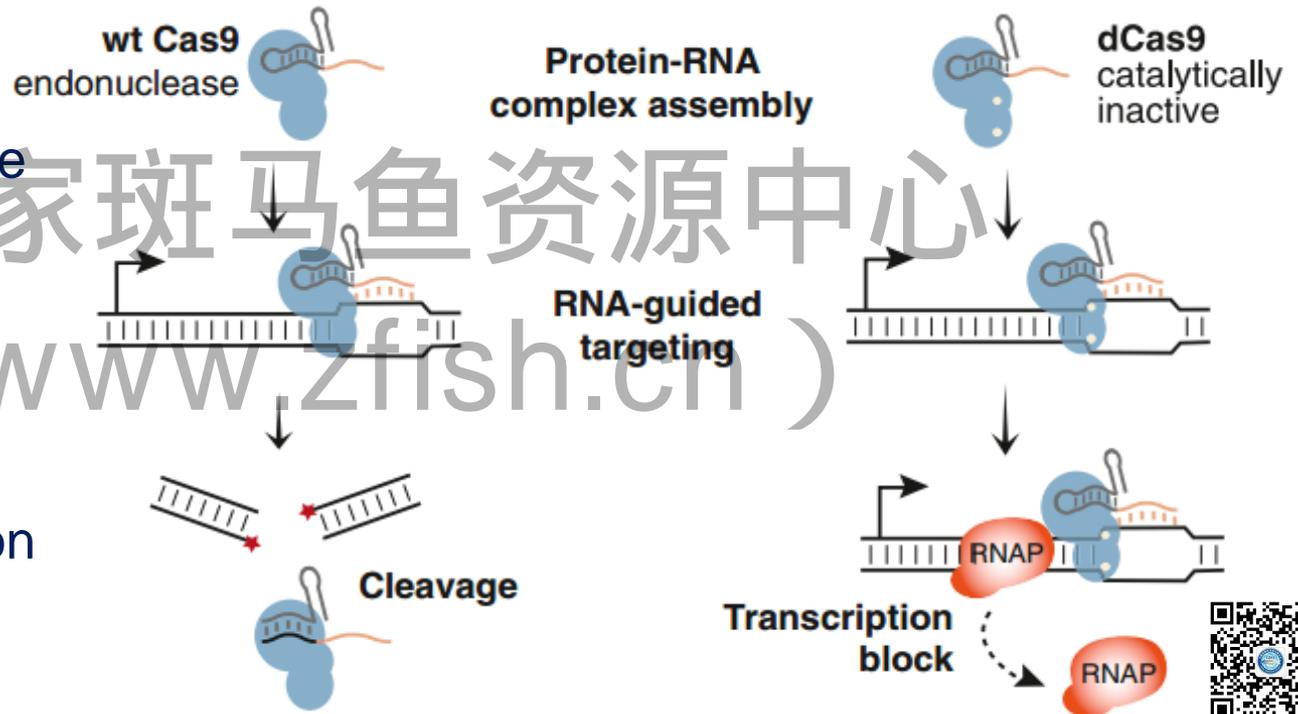


dCas9: dead Cas9

CRISPRi:

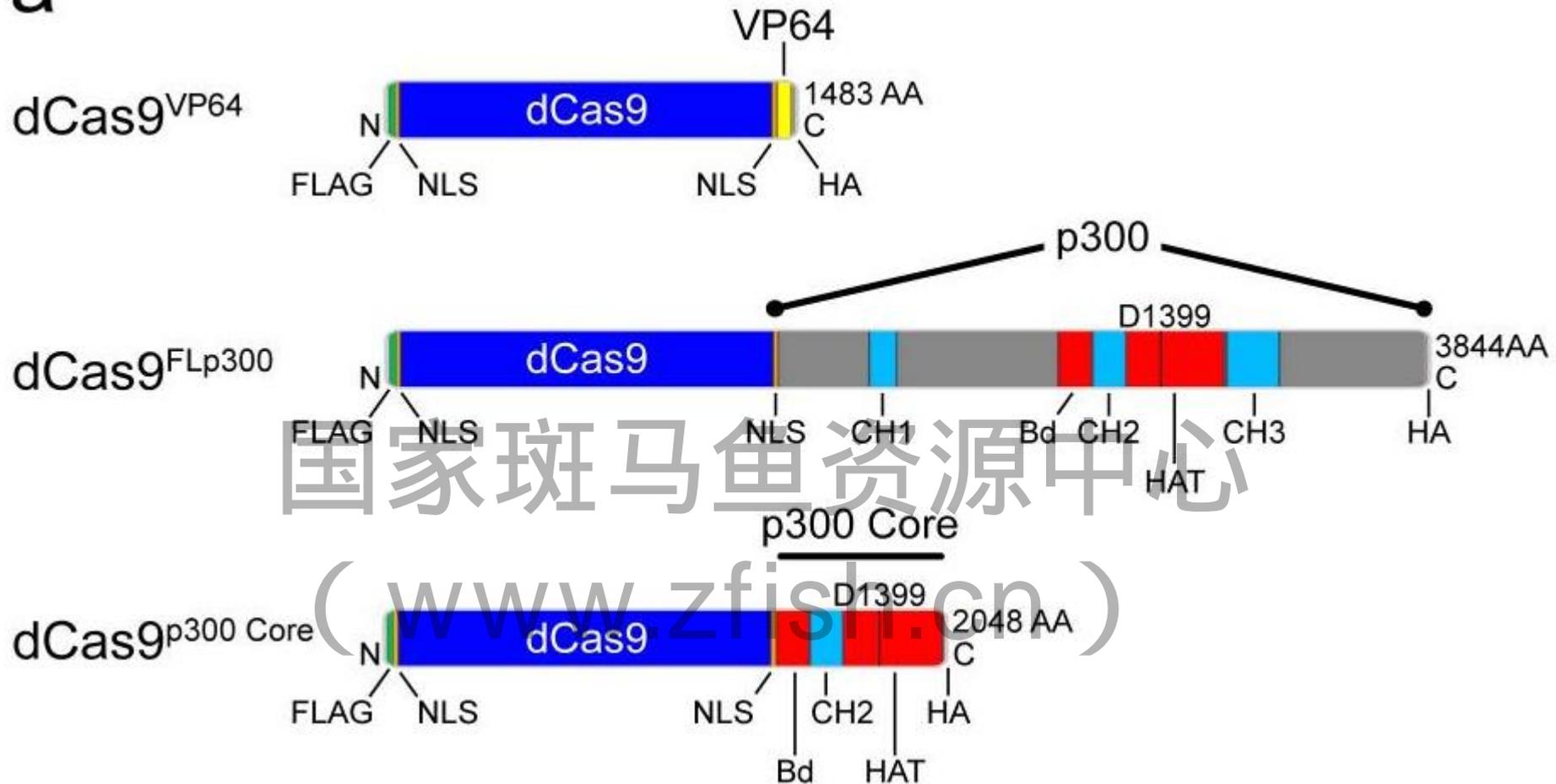
CRISPR interference

- On promoter:
Block initiation of transcription
- On coding sequence:
Block transcription elongation

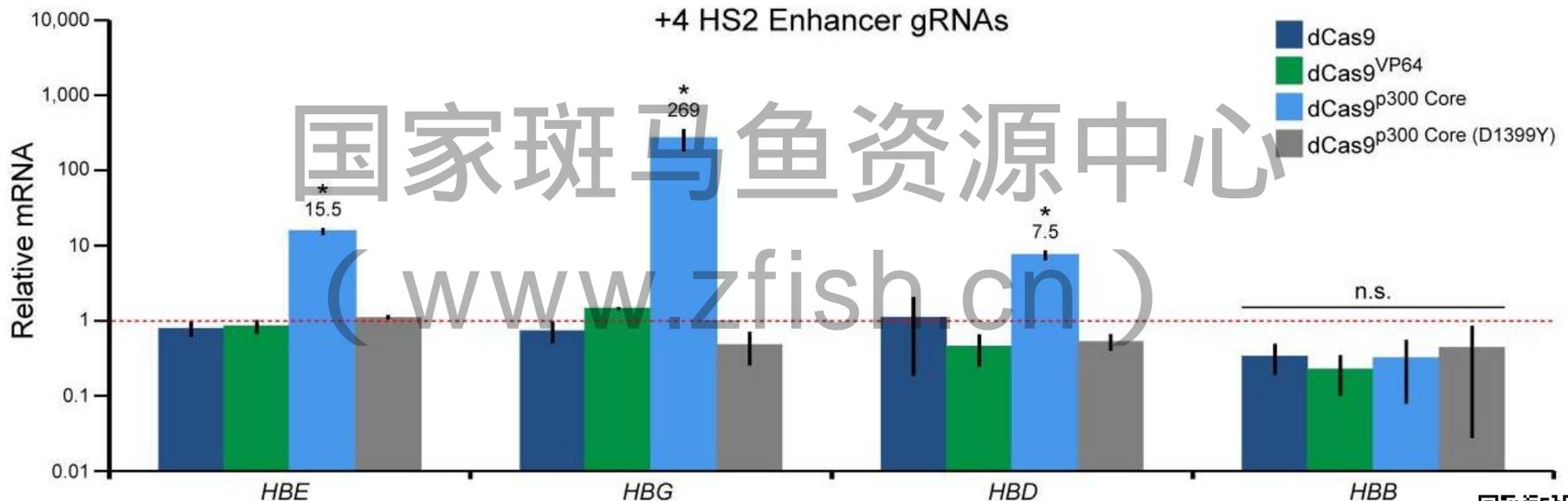
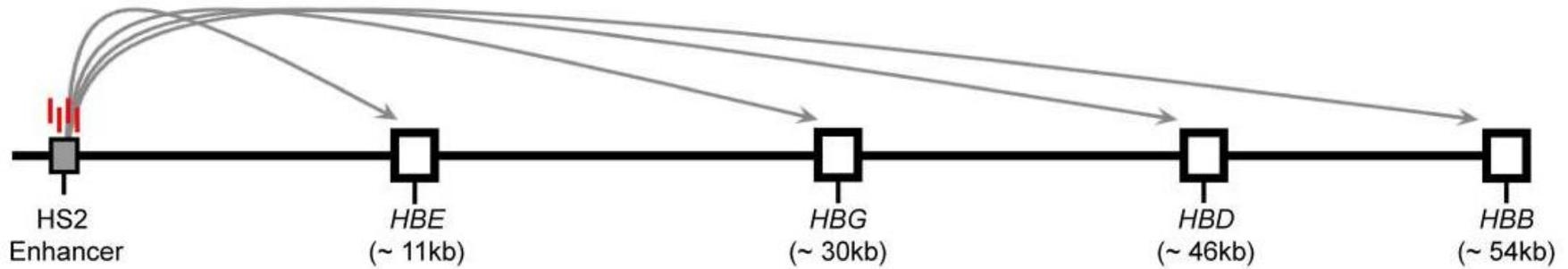


Modulating Gene Expression: epigenetic modification

a



dCas9-p300 fusion protein: catalyzes histone acetylation

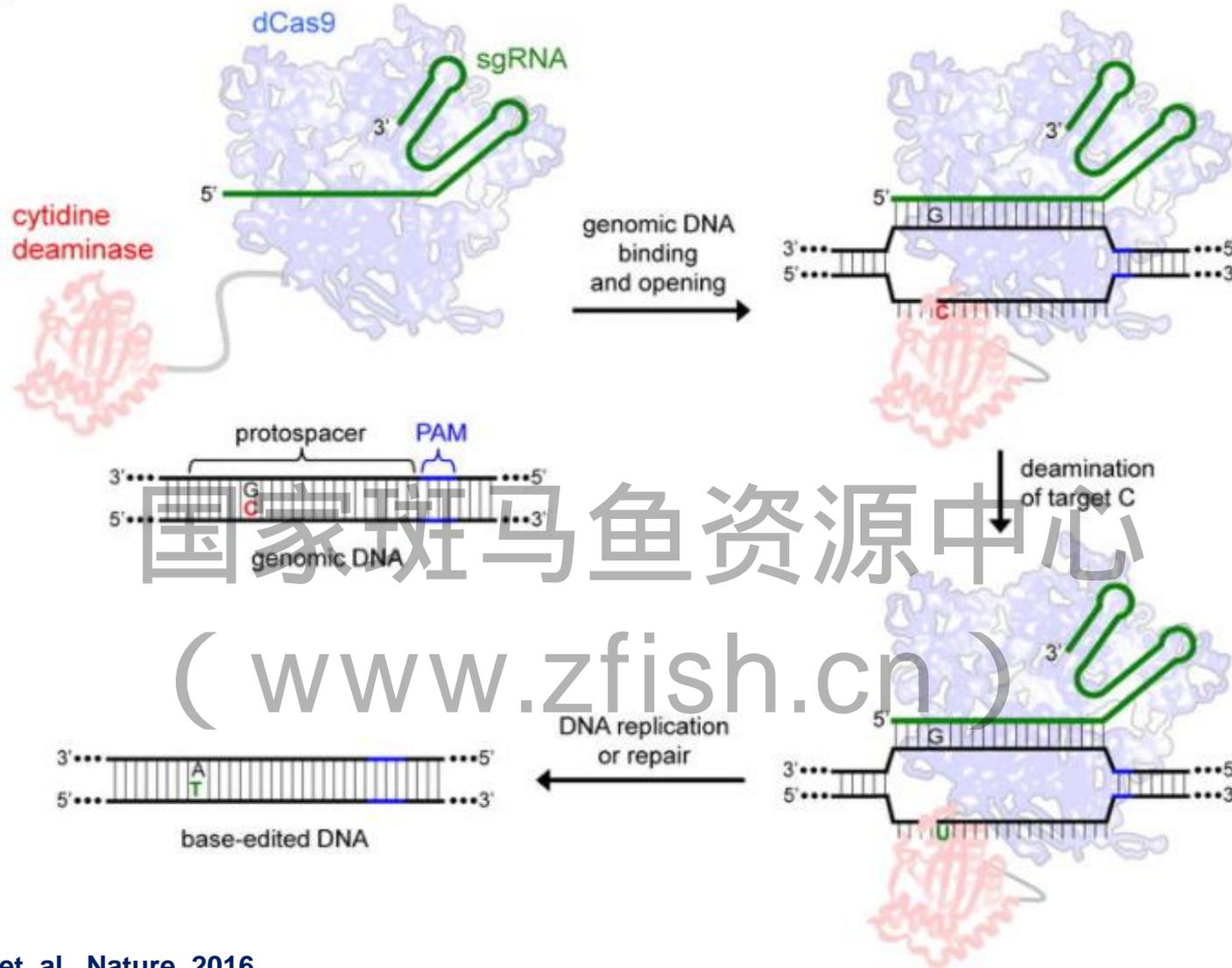


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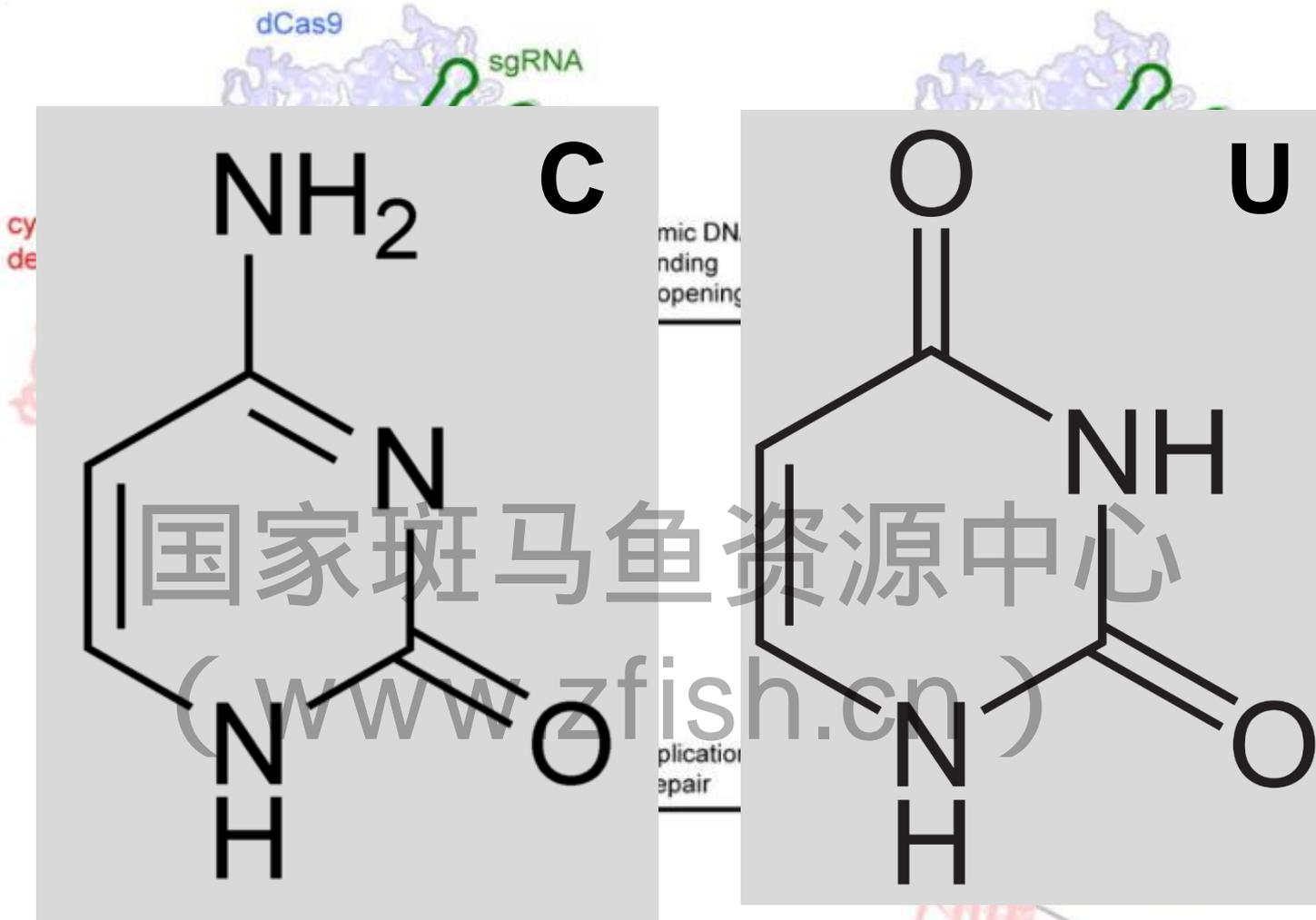
BE1 mediates C→U conversion



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BE1 mediates C→U conversion



Base Editing of Zebrafish Genome

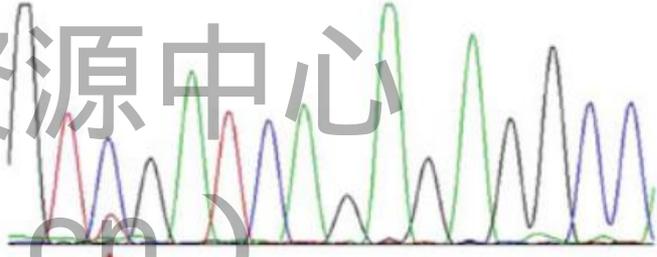
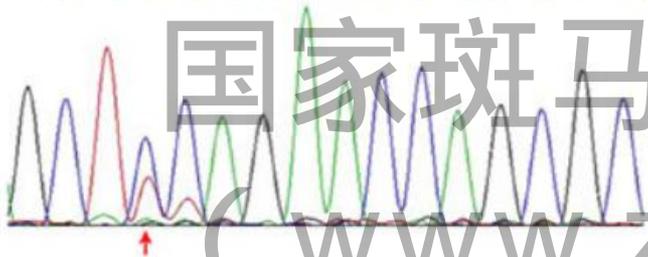
Target site	Sequence
<i>twist2-1</i>	GCT <u>CC</u> AGAACCAGCGCGTCCTGG
<i>twist2-2</i>	GCCG <u>CTC</u> GCGTACGTTCGCCAGG
<i>gdf6</i>	GT <u>CG</u> ATCAGAGAGGCCACTGG
<i>ntl</i>	GGA <u>ACC</u> AGCCACCGACTGTTGG

twist2-1

gdf6

G C T C C A G A A C C A G C G C

G T C G A T C A G A G A G G C C



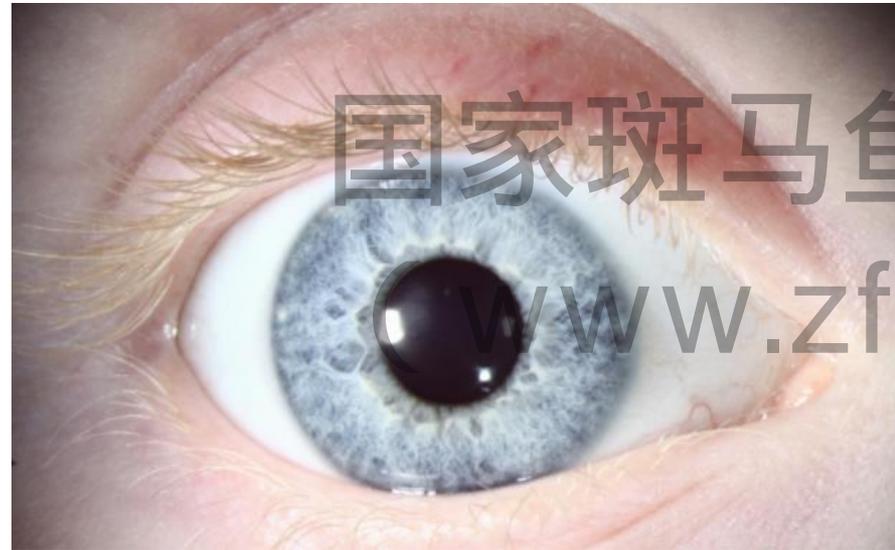
国家斑马鱼资源中心
www.zfish.cn

WT	GCTCCAGAACCAGCGCGTCCTGG	15/20
C->T	GCTTCAGAACCAGCGCGTCCTGG	3/20
	GCTTTAGAACCAGCGCGTCCTGG	1/20
C->A	GCTACAGAACCAGCGCGTCCTGG	1/20

WT	GTCGATCAGAGAGGCCACTGG	14/20
C->T	GTTGATCAGAGAGGCCACTGG	1/20
C->A	GTAGATCAGAGAGGCCACTGG	4/20
INDEL	-T --- T- A -----CACTGG	1



Human Disease Model: oculocutaneous albinism type IA



oculocutaneous albinism type IA (OCA1A):

- caused mutations in the tyrosinase gene (*tyr*)
- decreased or absent pigmentation in the hair, skin, and eyes
- decreased pigment in the iris and retina
- decreased visual acuity, misrouting of the optic nerves at the chiasm, and nystagmus



Base Editing of Zebrafish Genome

P301L

human cgt aat **cct** gga aac cat gac

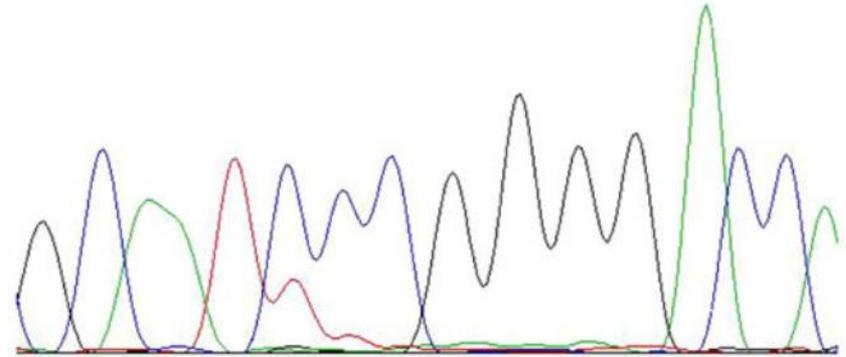
R N P G N H D

R N P G D H D

zebrafish cgc aat **ccc** ggg gac cac gac

P302L

G C A A T C C C G G G G A C C A

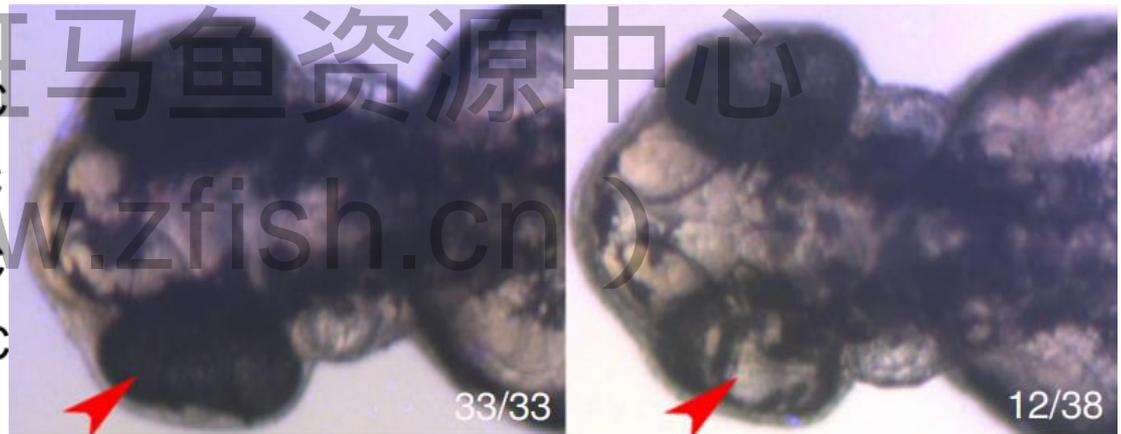


WT GC AAT CCC GGG GAC

C->T GC AAT TCC GGG GAC

C->A GC AAT ACC GGG GAC

C->G GC AAT GCC GGG GAC



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CRISPR/Cas in zebrafish

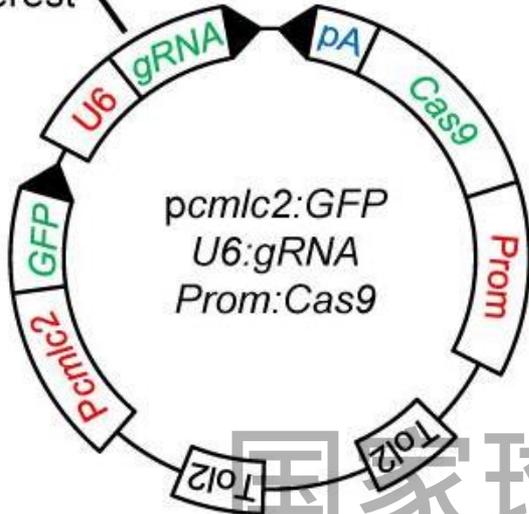
- Targeted Knock-out
- Targeted Knock-in
- Conditional inactivation
- Multigenic mutagenesis

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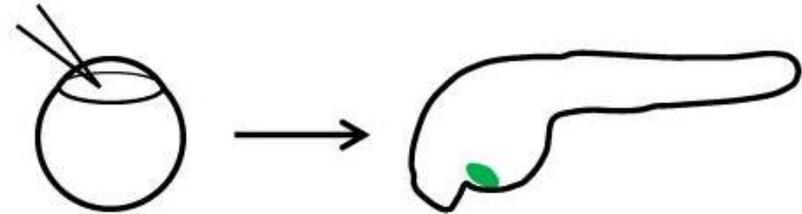
Conditional inactivation

Any target gene of interest



B

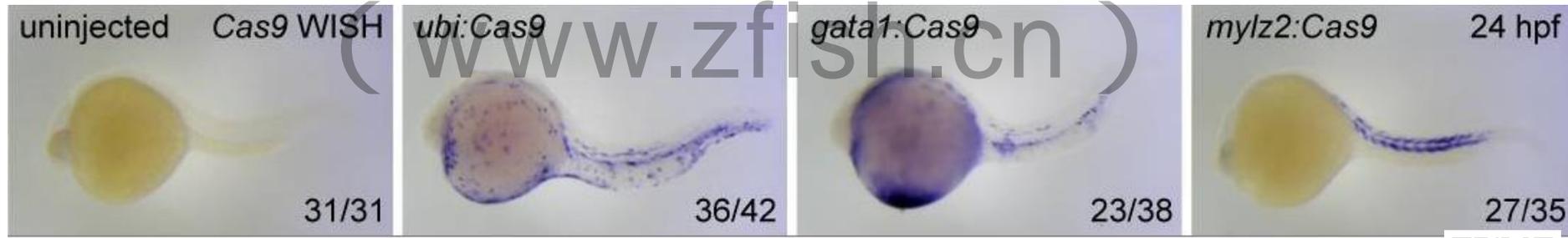
pcmlc2:GFP, *U6:gRNA*, *Prom:Cas9*
+ *Tol2* mRNA



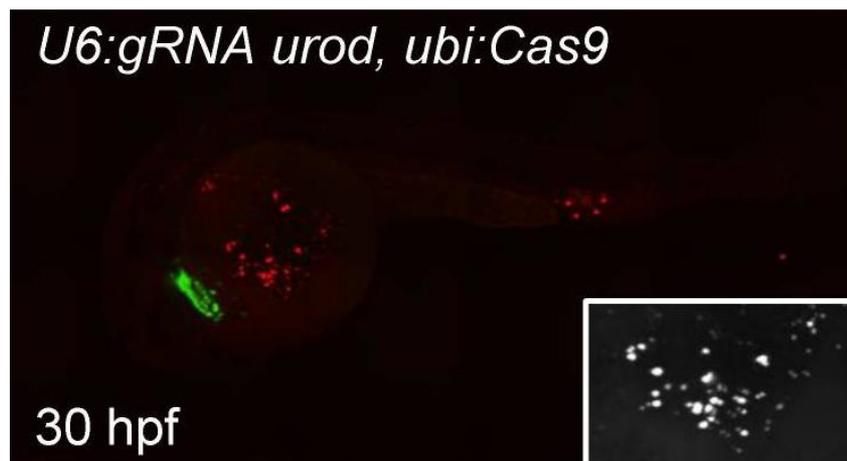
Any tissue-specific promoter

- fluorescence imaging
- *in situ* hybridization
- T7E1 assay
- sequencing

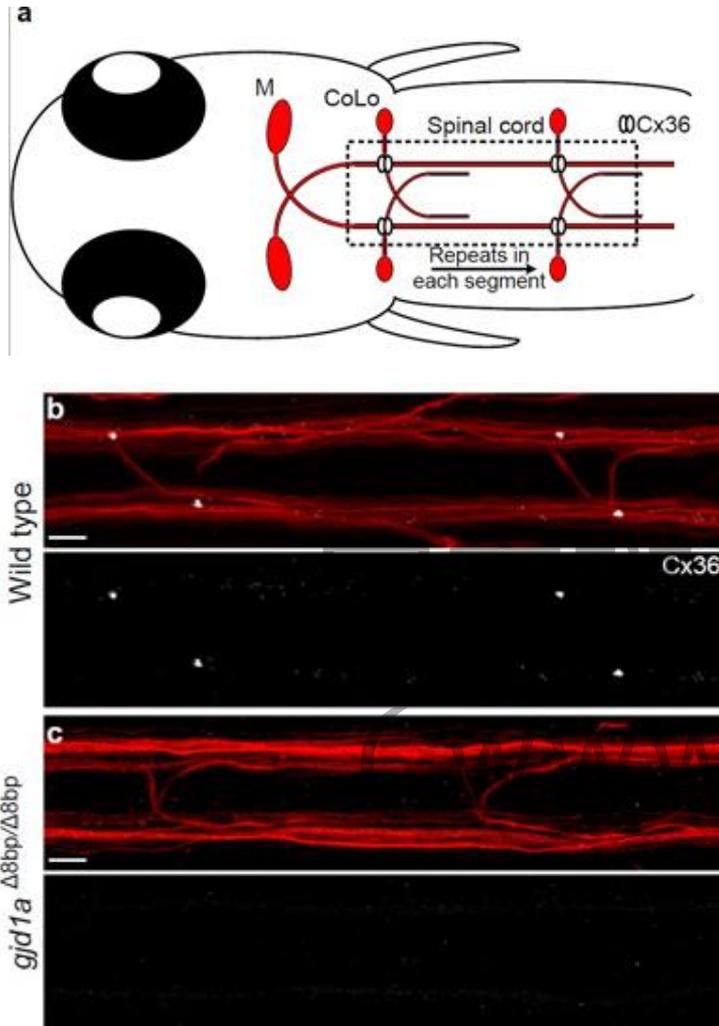
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Conditional inactivation



Multigenic mutagenesis

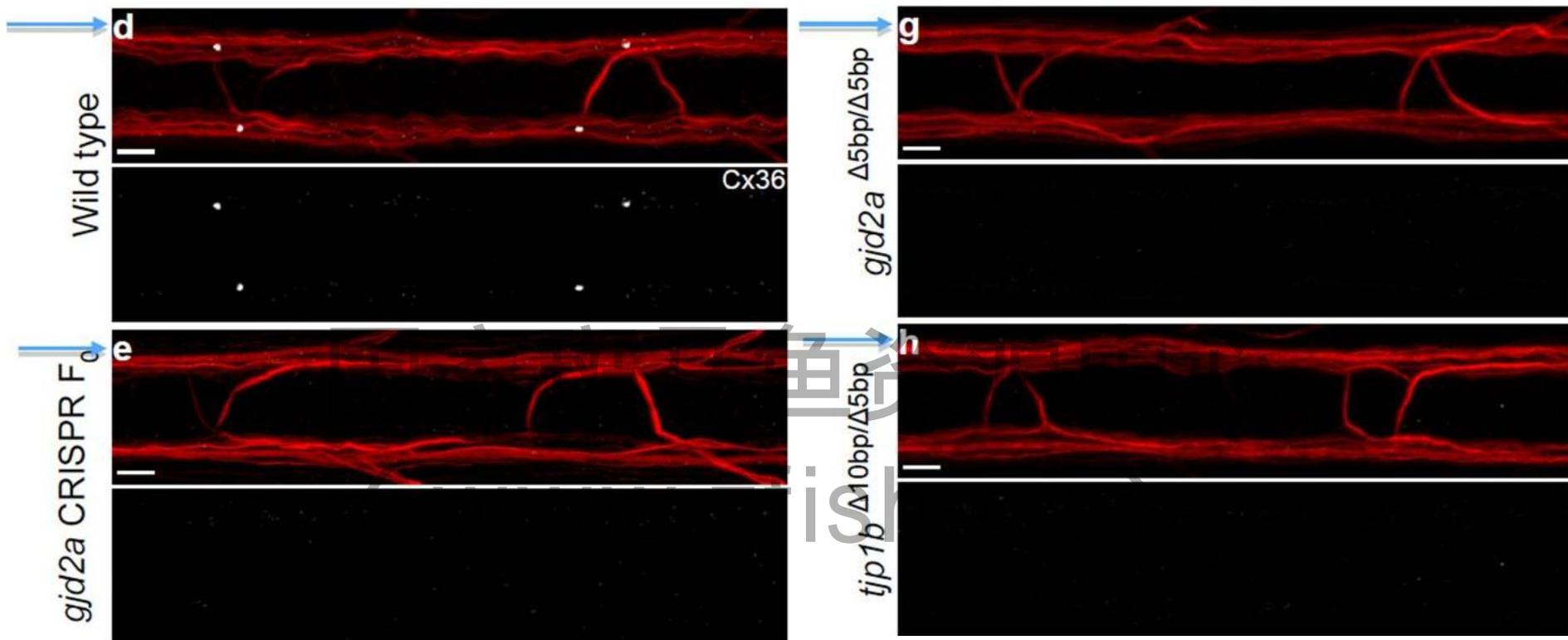


a

	1	2	3	4	5	6
A	<i>gjd1a</i>	<i>gjd2a</i>	<i>gjd2b</i>	<i>gjd1b</i>	<i>cntn1a</i>	<i>cntnap1</i>
B	<i>cnstb</i>	<i>cnsta</i>	<i>nlgn1</i>	<i>tjp1a</i> Long	<i>cntn1b</i>	<i>cntnap2</i>
C	<i>nrxn1a</i> Short	<i>nrxn1a</i> Long	<i>nlgn2a</i>	<i>tjp1a</i> Both	<i>cntn2</i>	<i>cntnap2a</i>
D	<i>nrxn1b</i> Short	<i>nrxn1b</i> Long	<i>nlgn2b</i>	<i>tjp1b</i> Long	<i>cntn3a</i>	<i>cntnap2b</i>
E	<i>nrxn2a</i> Short	<i>nrxn2a</i> Long	<i>nlgn3a</i>	<i>tjp1b</i> Both	<i>cntn3b</i>	<i>cntnap4</i>
F	<i>nrxn2b</i> Short	<i>nrxn2b</i> Long	<i>nlgn3b</i>	<i>tjp2a</i>	<i>cntn4</i>	<i>cntnap5</i>
G	<i>nrxn3a</i> Short	<i>nrxn3a</i> Long	<i>nlgn4a</i>	<i>tjp2b</i>	<i>cntn5</i>	<i>cntnap5a</i>
H	<i>nrxn3b</i> Short	<i>nrxn3b</i> Long	<i>nlgn4b</i>	<i>tjp3</i>	<i>cntn6</i>	<i>cntnap5b</i>



Multigenic mutagenesis



DSB-induced Knock-in

	template	Size of homo-arm	insertion
Hruscha et al. 2013	ssoDN	41+49 nt	HA tag
Irion et al. 2014	linear plasmid	875 bp	SNP
Auer et al. 2014	linear plasmid	homology-independent	KaTA4
Hisano et al. 2015	linear plasmid	10/20/40 bp	GFP
Li et al. 2015	linear plasmid	298+671 bp	GFP
Armstrong et al. 2016	ssoDN	23/33/100 nt	SNP
Richardson et al. 2016	ssoDN	36 nt	SNP



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感谢大家到CZRC参观学习

中科院水生所

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