



生物育种专业·基因编辑技术课程

第二章：基因剪刀手——人工特异性核酸内切酶

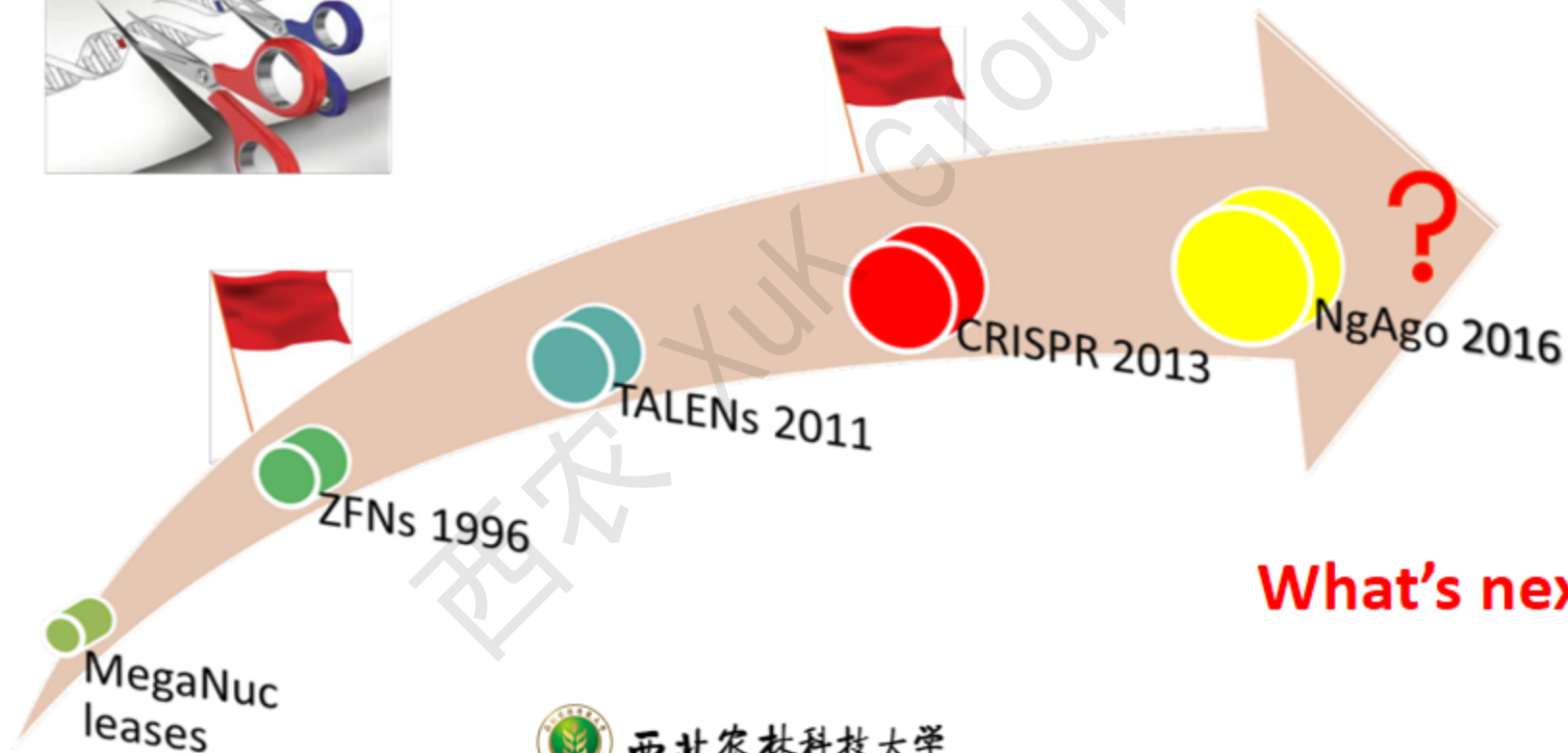
徐坤 副教授 QQ: 564737724 Tel:17792639752



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基因剪刀手—人工特异性核酸酶技术



What's next?



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目录

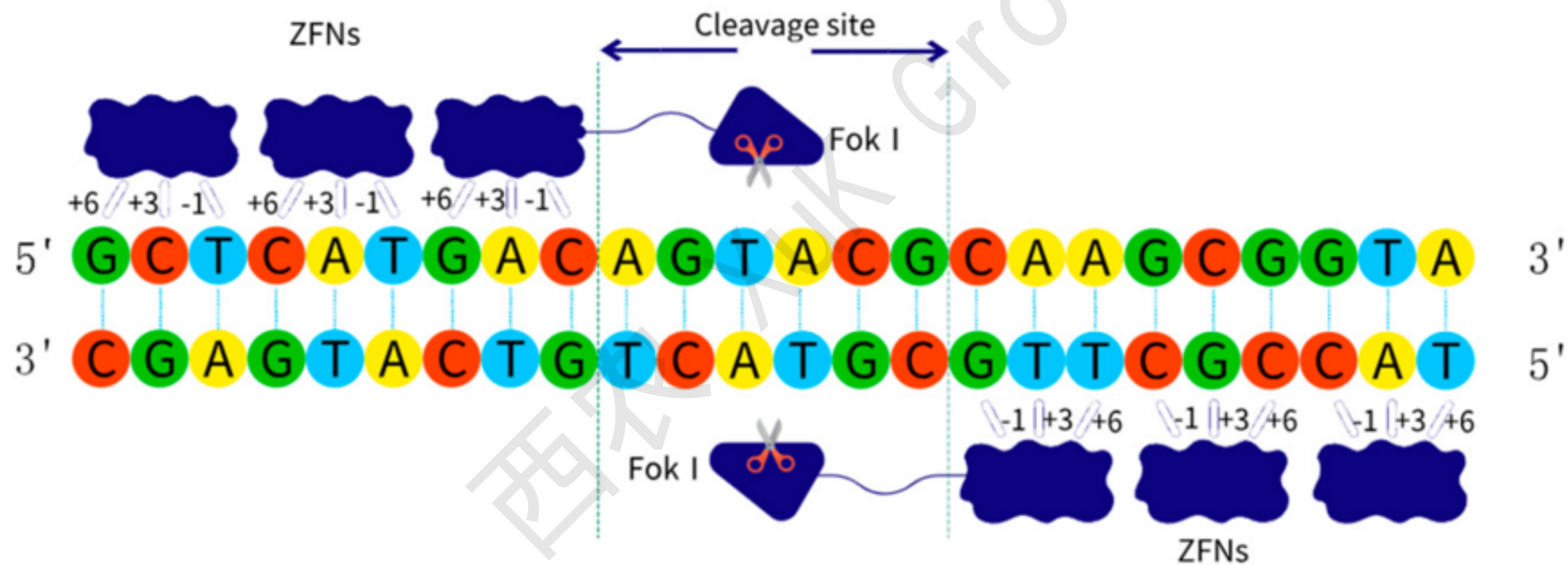
- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶



十年磨一剑：ZFNs

https://en.wikipedia.org/wiki/Zinc_finger_nuclease

锌指核酸酶：Zinc-Finger Nucleases (ZFNs)



> PLoS One. 2013 May 31;8(5):e64687. doi: 10.1371/journal.pone.0064687. Print 2013.

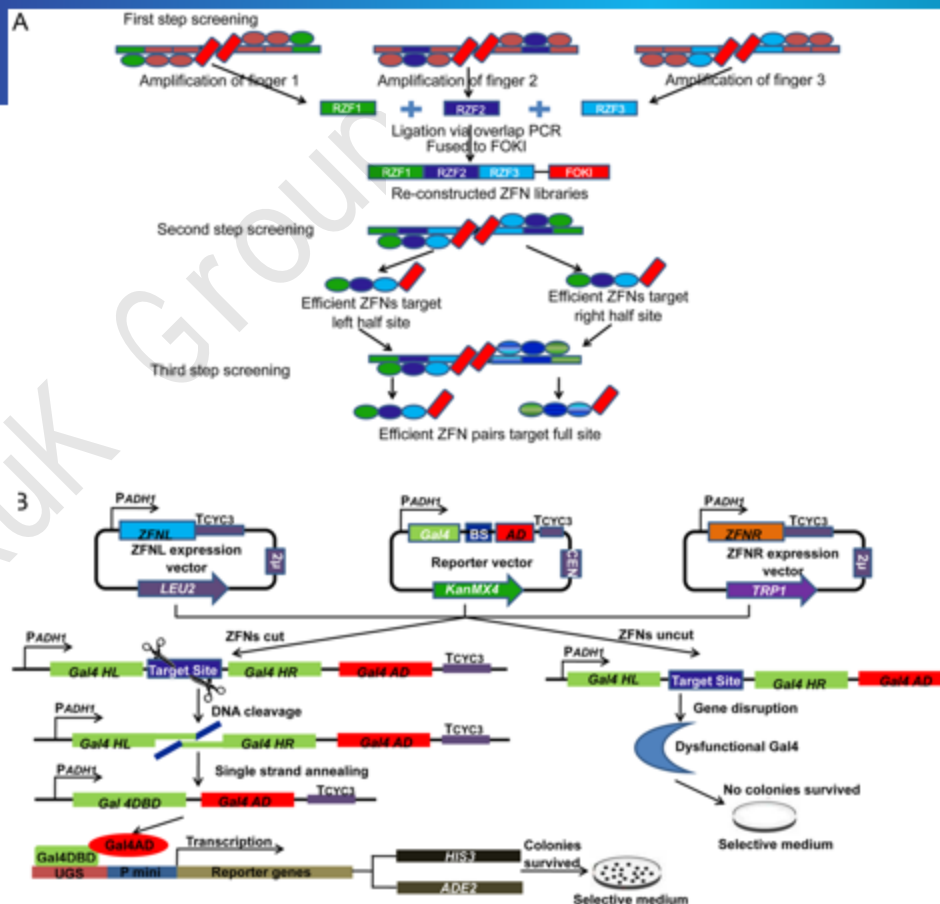
Simultaneous screening and validation of effective zinc finger nucleases in yeast

Ling Wang¹, Juan Lin, Tingting Zhang, Kun Xu, Chonghua Ren, Zhiying Zhang

Affiliations + expand

PMID: 23741369 PMCID: PMC3669427 DOI: 10.1371/journal.pone.0064687

<https://pubmed.ncbi.nlm.nih.gov/23741369/>



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目录

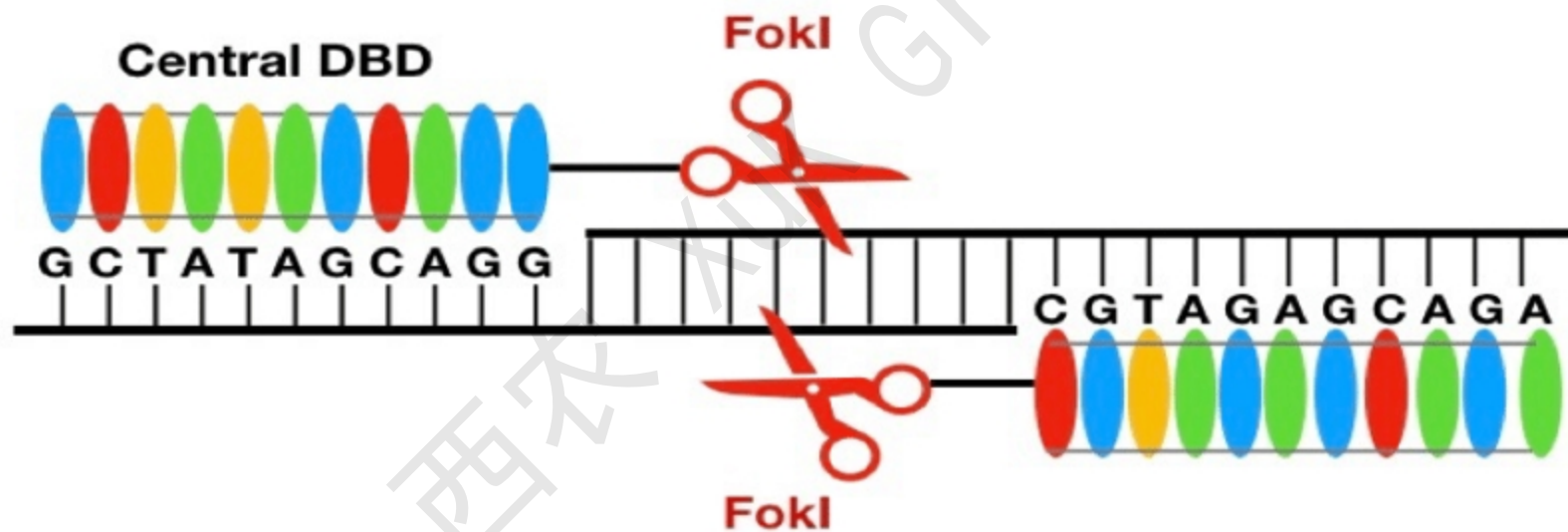
- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶



昙花一现：TALENs

https://en.wikipedia.org/wiki/Transcription_activator-like_effector_nuclease

Transcription Activator-Like Effector Nucleases (TALEN)



> PLoS One. 2013 Jun 20;8(6):e66459. doi: 10.1371/journal.pone.0066459. Print 2013.

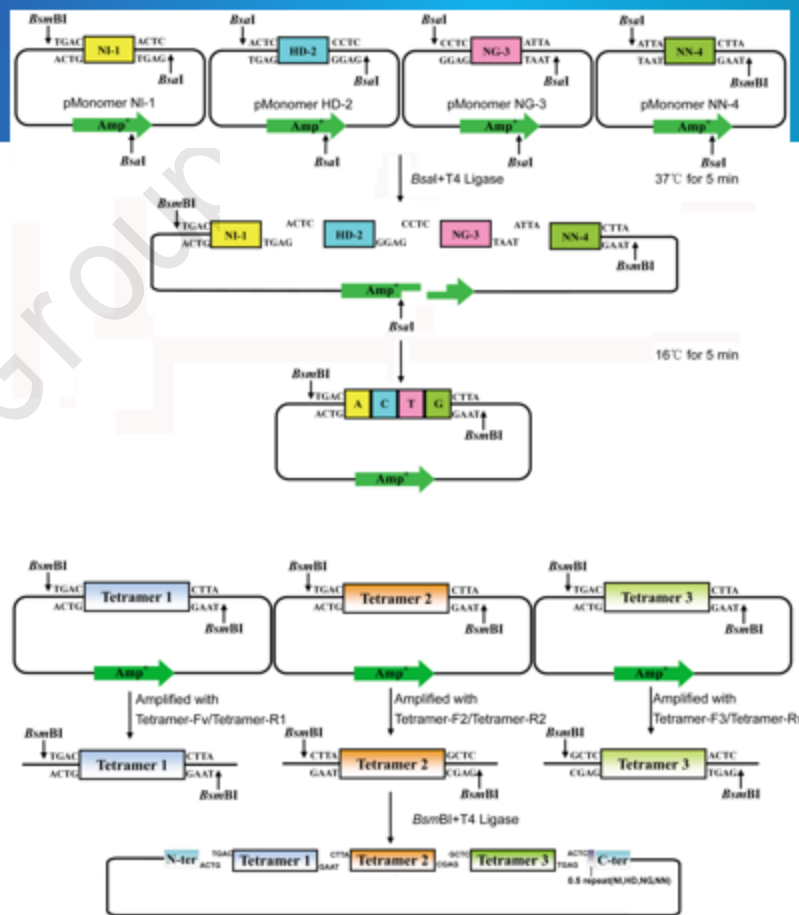
A simple and efficient method for assembling TALE protein based on plasmid library

Zhiqiang Zhang¹, Duo Li, Huarong Xu, Ying Xin, Tingting Zhang, Lixia Ma, Xin Wang, Zhilong Chen, Zhiying Zhang

Affiliations + expand

PMID: 23840477 PMCID: PMC3688977 DOI: 10.1371/journal.pone.0066459

<https://pubmed.ncbi.nlm.nih.gov/23840477/>



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目录

- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶



CRISPR/Cas9—改变世界的技术



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原核生物的「获得性」免疫系统

CRISPR:

Clustered Regularly Interspaced

Short Palindromic Repeats

规律间隔成簇短回文重复序列

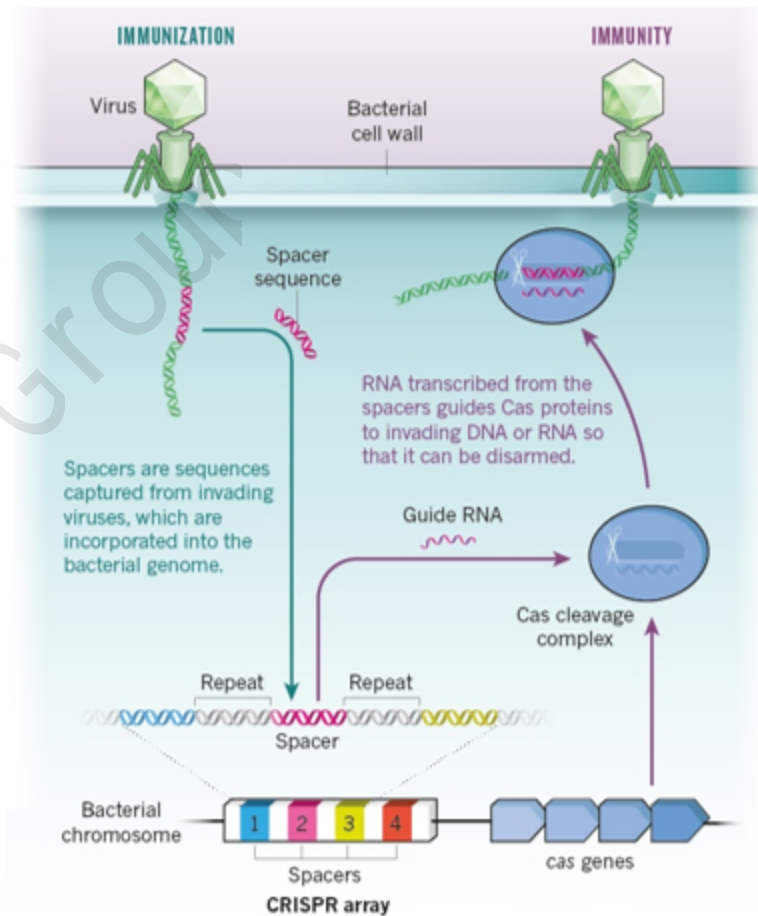
间隔序列 (Spacer)

重复序列 (Repeat)

Cas:

CRISPR关联基因 (CRISPR associated)

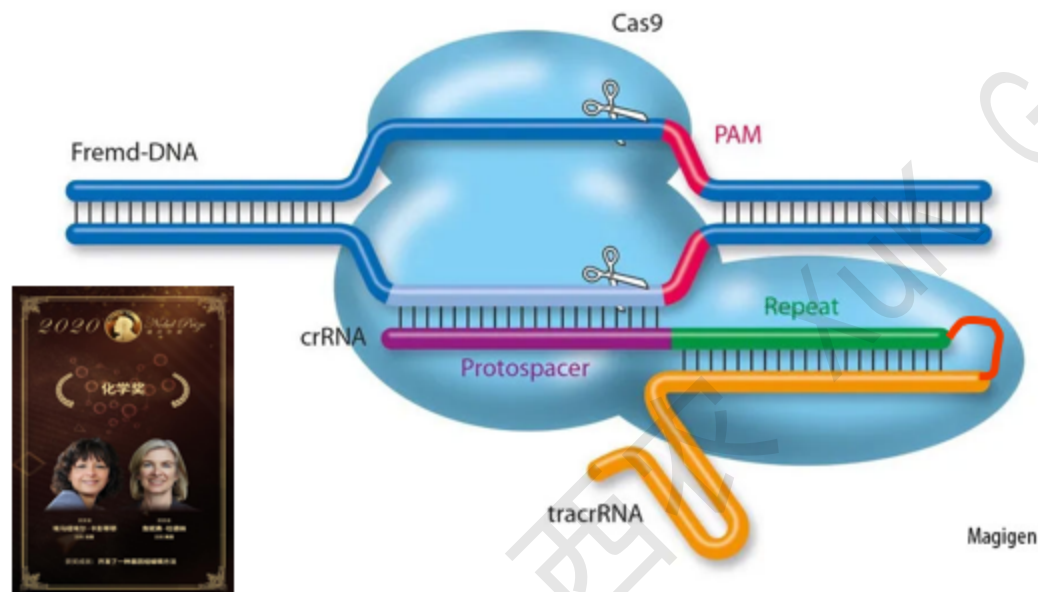
核酸内切酶(eg. Cas9)



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<https://pubmed.ncbi.nlm.nih.gov/22745249/>

CRISPR/Cas9技术

1. Cas9蛋白：

核酸内切酶, SpCas9, SaCas9

2. gRNA：

sgRNA = crRNA + tracrRNA

3. 靶点带PAM：

NGG → NGN、NRN (G/A)

4. gRNA/Cas9

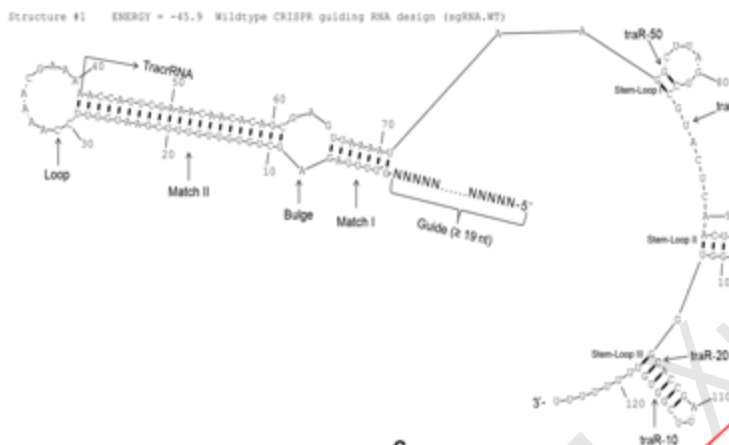
RGNs: RNA Guided Nucleases



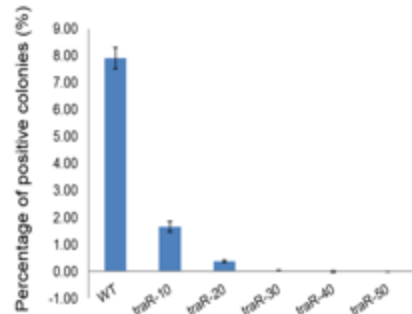
“我头上有犄角，我身后有尾巴”

<https://pubmed.ncbi.nlm.nih.gov/25038777/>

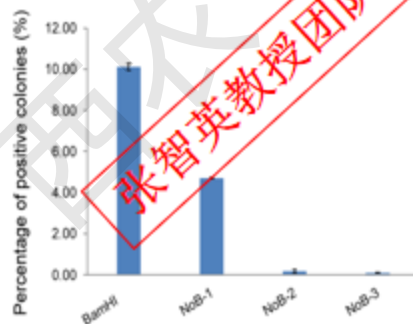
A



B



C



张智英教授团队

Cell. Mol. Life Sci. (2015) 72:383–399
DOI 10.1007/s00118-014-1679-z

Cellular and Molecular Life Sciences

RESEARCH ARTICLE

Efficient genome engineering in eukaryotes using Cas9 from *Streptococcus thermophilus*

Kun Xu · Chonghua Ren · Zhongtian Liu · Tao Zhang ·
Fengting Zhang · Duo Li · Ling Wang · Qiang Yan ·
Lijun Guo · Juncen Shen · Zhiying Zhang

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EFFICIENT GENOME ENGINEERING IN EUKARYOTES USING CAS9 FROM *STREPTOCOCCUS THERMOPHILUS*.

November 26, 2014

Kun Xu, Chonghua Ren, Zhongtian Liu, Tao Zhang, F. Zhang, Duo Li, Ling Wang, Q. Yan, L. Guo, J. Shen, Z. Zhang

Cell Mol Life Sci. 2014 Jul 26.

Key Laboratory of Molecular Biology of Chongqing University, Chongqing, China; Institute of Agricultural Biotechnology, North China University of Technology, Beijing, China

Abstract

The *Streptococcus thermophilus* CRISPR-Cas (Cas9) system has been shown to mediate DNA cleavage in its original host and in *S. cerevisiae* as well as in vitro. Here, we have characterized the Cas9 system in greater detail and conducted a systematic comparison of the optimal length of tracrRNA, loop structure, Match II region, and sequence position of the target sequence within the crRNA. Tolerance of mismatches and sequence position of the target sequence within the crRNA system achieved up to 12 and 40% larger tolerance, respectively, in *S. cerevisiae* and *Arabidopsis thaliana* cells, respectively. This study provides important insight into the tolerance and target requirements necessary to develop a targeted and highly efficient eukaryotic gene editing platform using Cas9 systems.

Go to PubMed

Significance Statement

KEY SCIENTIFIC ARTICLES

Efficient genome engineering in eukaryotes using Cas9 from *Streptococcus thermophilus*.
November 26, 2014

Quantum searching is an antibiocide strategy employed by endophytic bacteria.
November 26, 2014

SPY-ORF588A pioneering activity regulates promoter-specific recruitment of PRR4/Gammap.
November 26, 2014

Erythrokinase-mediated translation activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress.
November 26, 2014

Evidence for compartmentalization of iron-sulfur protein metabolism.
November 26, 2014

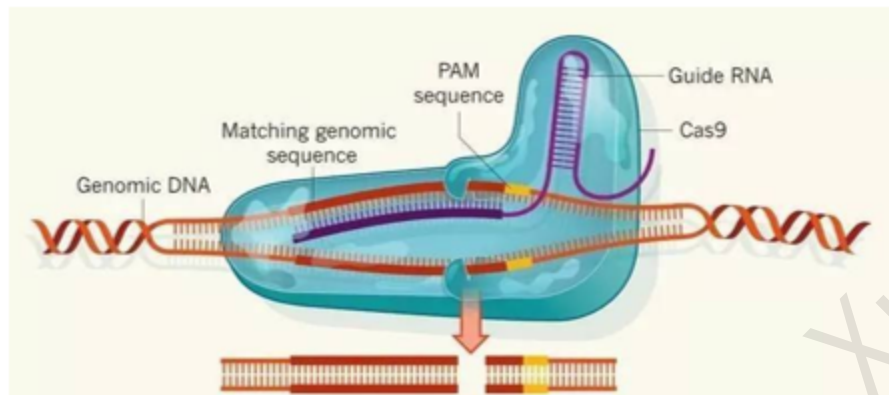
Establishment of auxin-induced dependent and independent heterologous gene expression in *Saccharomyces cerevisiae* NAG254.
November 26, 2014

Design and synthesis of polymeric hydrogen sulfide donors.
November 26, 2014

Long-distance communication between tergiteal carcinoma cells.
November 26, 2014

Characterizing the inhibitory actions

坏掉的剪刀—“nCas9”和“dCas9”



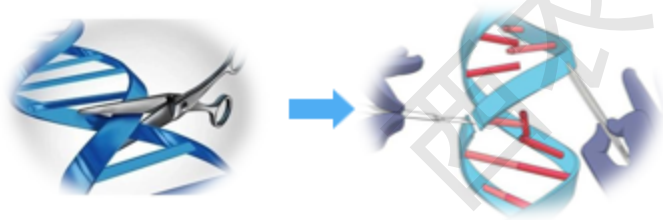
➤ 以CRISPR/Cas9为例

➤ **Cas9缺口酶** (nCas9, Cas9n) :

D10A or H840A

➤ **“死掉”的Cas9** (dead Cas9, dCas9):

D10A and H840A



A phoenix rising from flames over water. The background is a vibrant orange and red sunset or sunrise over a body of water. In the center, a phoenix is depicted with its wings spread, rising from a pool of fire. The overall scene is dramatic and symbolic of rebirth.

CRISPR/Cas9

之凤凰涅槃

dCas9

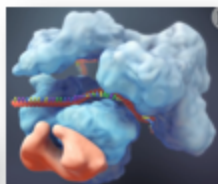
Cas9死了? 不,

他还活着!

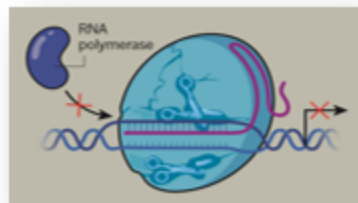
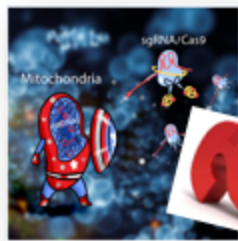
并且很精彩!

“八臂哪吒” -- 基于CRISPR/Cas9系统的衍生技术

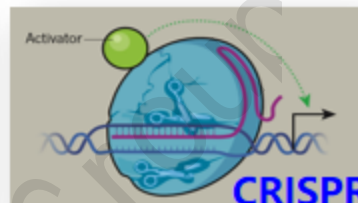
CRISPR gene editing



mtDNA editing
(TALE-BE)



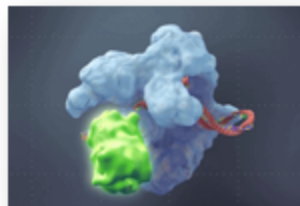
CRISPR interference (CRISPRi)



CRISPR activation (CRISPRa)



CRISPR imaging



CRISPR Screening



CRISPR detection

Cas12/DETECTR, Cas13/SHERLOCK



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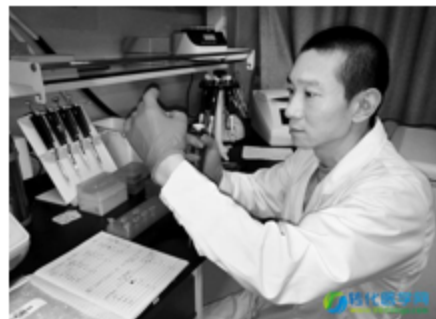
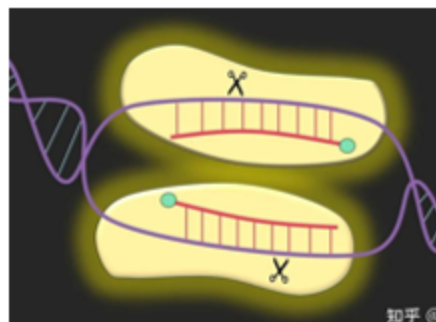
目录

- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶



黄梁成一梦：gDNA/NgAgo (pAgo)

<https://en.wikipedia.org/wiki/NgAgo>



[nature](#) > [nature biotechnology](#) > [articles](#) > [article](#)

Article | Published: 02 May 2016

DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute

[Feng Gao](#), [Xiao Z Shen](#), [Feng Jiang](#), [Yongqiang Wu](#) & [Chunyu Han](#)

Nature Biotechnology **34**, 768–773 (2016) | [Cite this article](#)

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- 1 A [Retraction](#) to this article was published on 01 August 2017
- 1 A [Retraction](#) to this article was published on 01 August 2017
- 1 An [Addendum](#) to this article was published on 28 November 2016
- 1 This article has been [updated](#)

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Seung Hwan Lee, Giandomenico Turchiano ... Jin-Soo Kim
Nature Biotechnology | **Correspondence** | 28 Nov 2016

Sections

Figures

References

[Abstract](#)

[Accession codes](#)

[Change history](#)



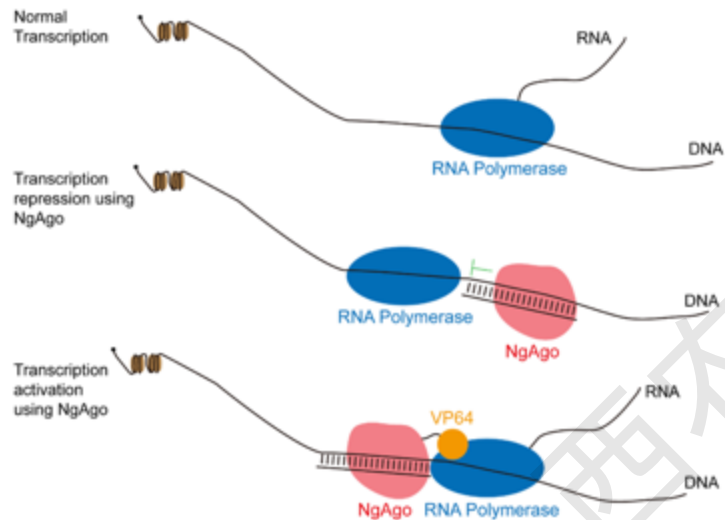
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<https://www.nature.com/articles/nbt.3547>

“东方不亮西方亮，黑了南方有北方”

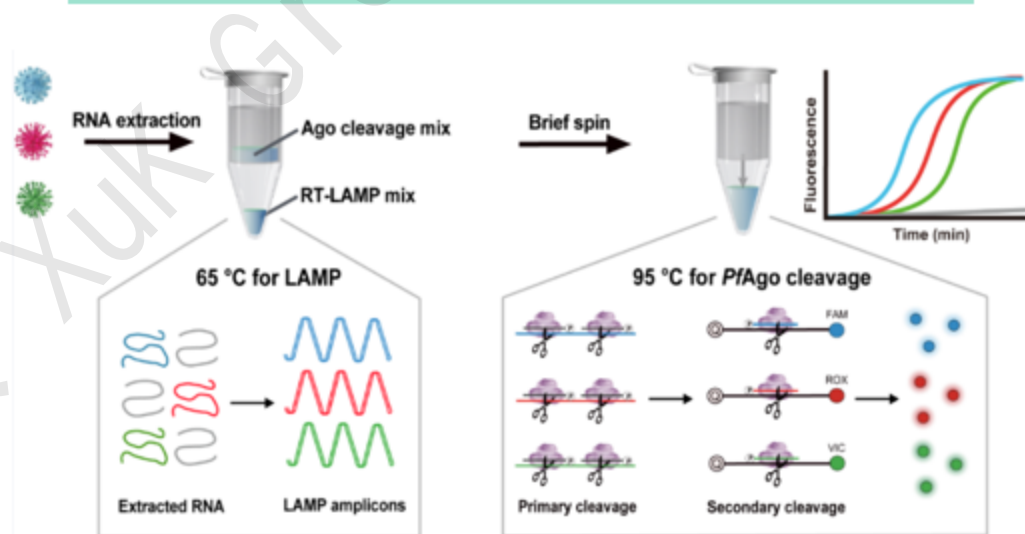
AGOi & AGOa

Transcription Manipulation using NgAgo



AGO Detection

MULAN (Multiplex Argonaute-based Nucleic Acid Detection)



<https://pubmed.ncbi.nlm.nih.gov/35334329/>



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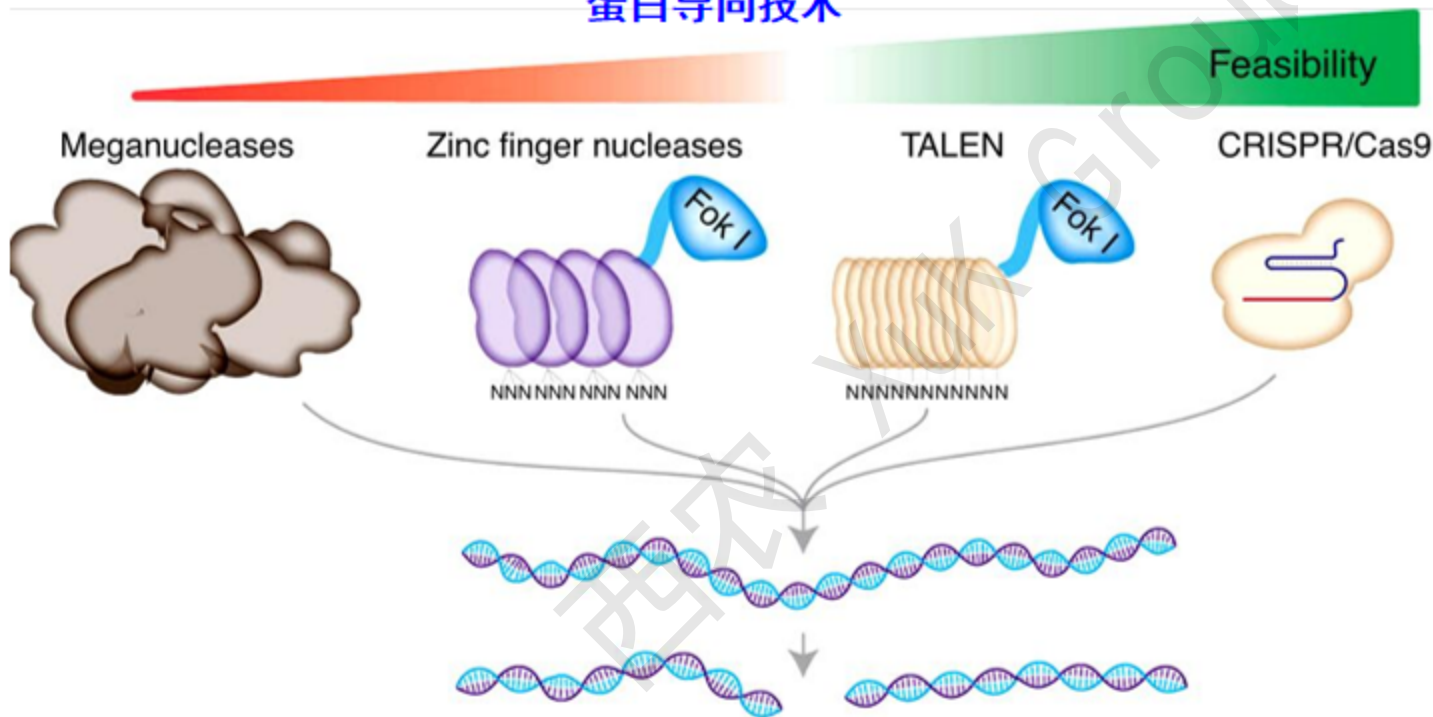
目录

- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶



基因剪刀手--人工特异性核酸酶技术

蛋白导向技术



RNA导向技术:

CRISPR/Cas9系列
CRISPR/Cas12系列
OMEGA/IscB系列
OMEGA/TnpB系列
OMEGA/Fanzor系列

HYER: Hydrolytic
endonucleolytic ribozyme

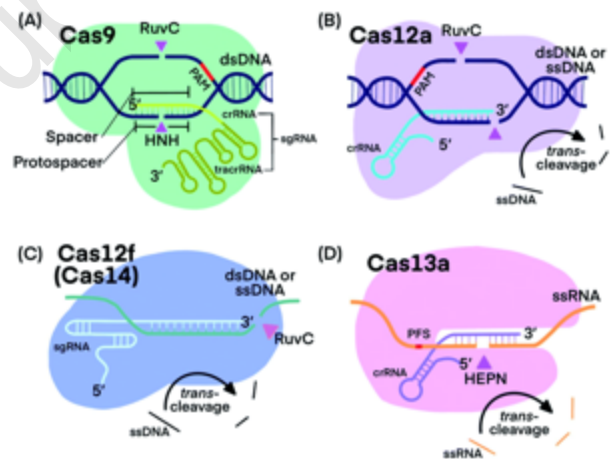
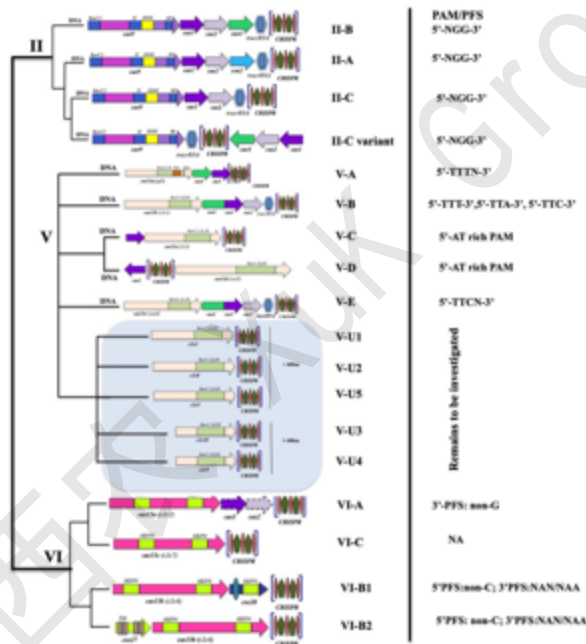
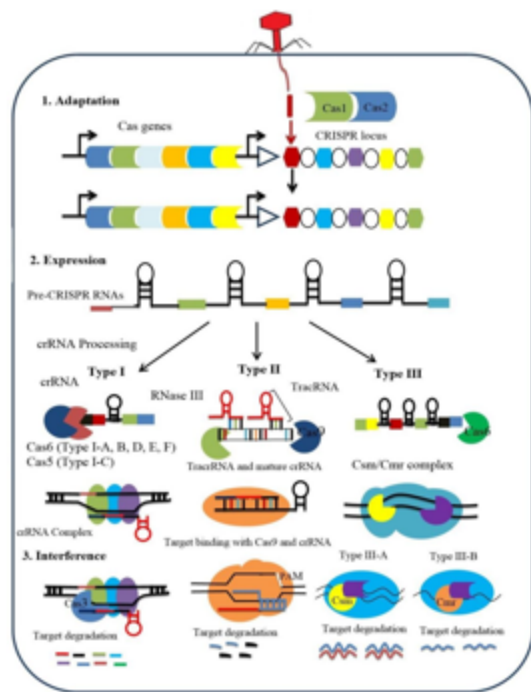
DNA导向技术?

gDNA/NgAGO × × ×
韩春雨事件



西北农林科技大学

丰富多样的CRISPR基因剪刀手



张锋

Jennifer A. Doudna



各式各样的CRISPR/Cas9系统

CRISPR-Cas工具酶介绍 (qq.com)

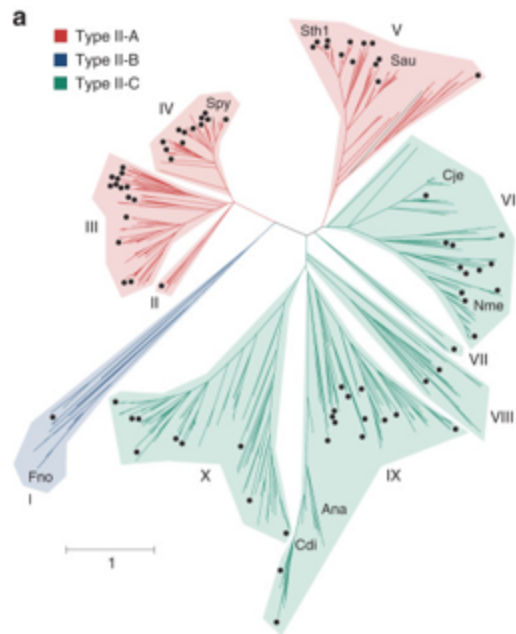


表2. 本实验室筛选的Cas9工具酶

名字	Type II	大小	PAM	活性	特异性	时间
SauriCas9	A	1061	NNGG	高	中	2020.3
SlugCas9	A	1054	NNGG	高	低	2021.4
SlugCas9-HF	A	1054	NNGG	高	高	2021.4
ShaCas9	A	1055	NNGGV	中	中	2021.4
SlurCas9	A	1054	NNGRR	中	中	2021.4
SchCas9	A	1054	NNGR	中	中	2022.2
Sha3Cas9	A	1055	NNGRC	高	中	2022.2
Nisp2Cas9	C	1067	NNNCC	中	高	2022.8
NarCas9	C	1070	MNNC	低	-	2022.8
Sha2Cas9	A	1058	NNGG	高	中	2022.11
Sha2Cas9-HF	A	1058	NNGG	高	高	2022.11
SpeCas9	A	1058	NNGG	高	中	2022.11
SpeCas9-HF	A	1058	NNGG	高	高	2022.11
SmiCas9	A	1063	NNGG	中	高	2022.11
Hsp1Cas9	C	1057	NNNRRAA	中	低	2023.4
Hsp2Cas9	C	1067	NNNCC	中	低	2023.4
CcuCas9	C	1042	NNNCCA	中	低	2023.4

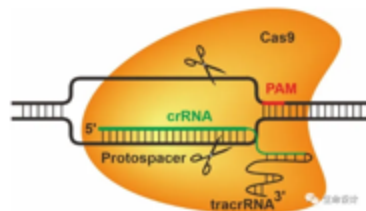
有的Cas9识别独特的PAM，但是活性不高，将识别PAM的结构域替换到活性/特异性高的同系物中，得到的嵌合体Cas9性能更好(表3)

表3. Cas9嵌合体工具酶

名字	Type II	大小	PAM	活性	特异性	时间
Sa-SlugCas9	A	1055	NNGG	高	高	2021.4
Sa-SchCas9	A	1055	NNGR	中	中	2022.2
Nisp2-SmiCas9	C	1072	MNNC	中	高	2022.8
Hsp1-Hsp2Cas9	C	1048	MNNCY	中	低	2023.4
Hsp1-Hsp2Cas9-Y	C	1048	MNNCY	中	高	2023.4
Hsp1-Hsp2Cas9-KY	C	1048	MNNCY	中	高	2023.4

表1. Cas9工具酶

名字	Type II	大小	PAM	活性	特异性	国家	时间
SpCas9	A	1368	NNG	高	低	美国	2013.2
St1Cas9	A	1121	NNAGAAW	低	中	美国	2013.2
NmCas9	C	1082	NNNGATT	低	高	美国	2013.8
St3Cas9	A	1409	NNGNG	中	高	中国	2015.1
SaCas9	A	1053	NNGRRT	高	高	美国	2015.4
FnCas9	B	1629	NNG	低	中	美国	2016.2
CjCas9	C	984	NNNRVAC	低	高	韩国	2017.2
GeoCas9	C	1087	NNNCRAA	低	低	美国	2017.11
ScCas9	A	1380	NNG	低	低	美国	2018.10
Nme2Cas9	C	1082	NNNCC	中	高	美国	2019.2



复旦大学王永明

<https://pubmed.ncbi.nlm.nih.gov/33139742/>



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各式各样的CRISPR/Cas12系统

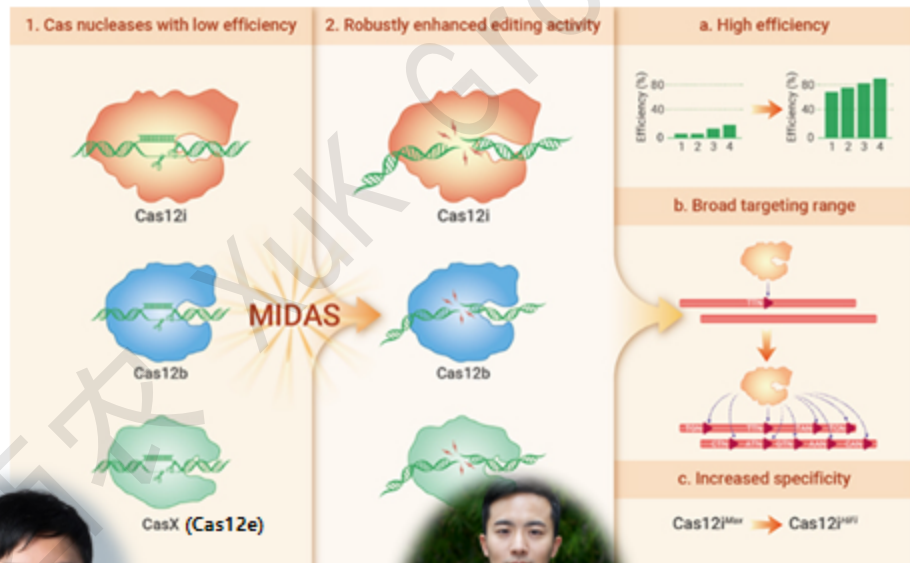
表4. Cas12工具酶

名字	大小	PAM	活性	特异性	时间
AsCas12a	1307	TTTV	高	高	2015.9
LbCas12a	1228	TTTV	中	高	2015.9
FnCas12a	1300	TTN	低	高	2015.9
Mb3Cas12a	1261	TTN	中	-	2020.9
TsCas12a	1298	TTN	中	-	2020.9
Mb2Cas12a	1251	TTN	中	-	2020.9
BsCas12a	1206	TTN	高	-	2020.9
PiCas12a	1323	KKYY	高低	-	2020.6
HkCas12a	1310	YYV	低	-	2020.6
CeCas12a	1287	TTTV	中	高	2020.3
BfCas12a	1231	TTTV	中	-	2020.3
Cas12a-M29-1	1280	YYN	高	-	2020.12
Lb2Cas12a	1206	TTN	中	-	2021.12
PrCas12a	1213	TTTV	高	-	2021.12
PxCas12a	1215	TTN	中	-	2021.12
PdCas12a	1323	TTTV	低	-	2021.12
MbCas12a	1373	TTV	低	-	2021.12
EeCas12a	1282	TTTT	中	-	2021.12
ArCas12a	1266	TTN	低	-	2021.12
ErCas12a	1263	YTTN	中	-	2021.12
AaCas12b	1129	TTN	中	高	2018.11
BhCas12b v4	1108	RTTN	高	高	2019.1
BvCas12b	1112	ATTN	低	-	2019.1
DpbCas12e	986	TTCN	中	-	2019.2
PtmCas12e	978	TTCN	中	-	2022.2
Cas12 ^{MW}	1054	HNN	高	低	2022.5
Cas12j-8	718	TTN	高	极高	2023
AsCas12f1	422	TTR	低	高	2021
Un1Cas12f1	529	TTTT	中	极高	2021
enOsCas12f1	433	TTH	高	高	2021
enRhCas12f1	415	CCD	高	高	2021

中国农大赖锦盛



中科院神经所杨辉



上科大季泉江



清华大学刘俊杰



中科院动物所李伟

更多的“剪刀手”

中科院动物所
王皓毅



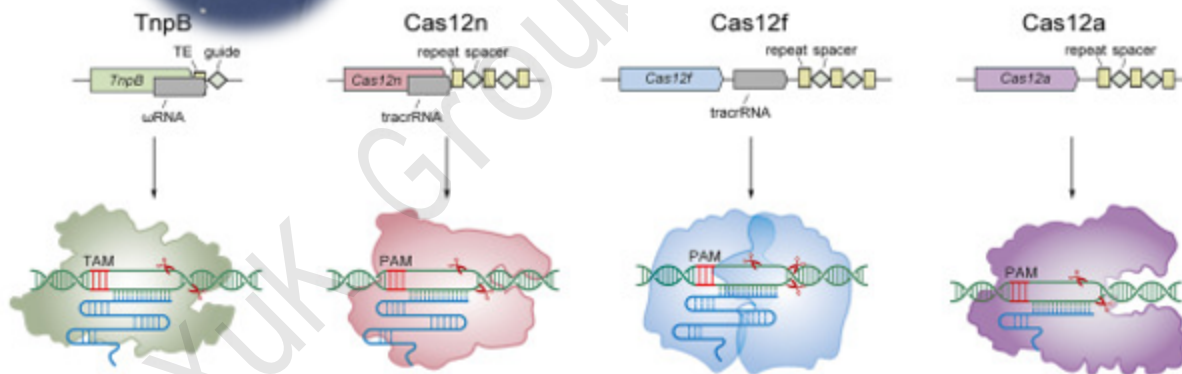
立陶宛
virginijus siksnys



CRISPR:

Clustered Regularly Interspaced Short Palindromic Repeats

规律间隔成簇短回文重复序列



OMEGA :

Obligate Mobile Element Guided Activity

具有导向活性的指定移动元件

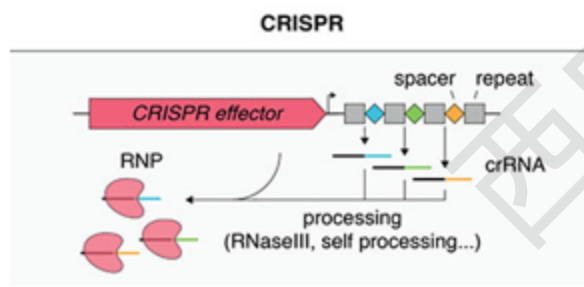
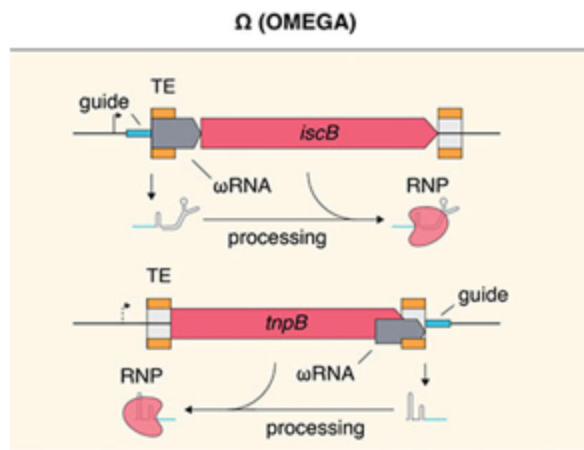
System	IS200/IS605 and IS607	CRISPR-Cas	CRISPR-Cas	CRISPR-Cas
Protein	~400 aa (monomer)	400-700 aa (likely monomer)	400-700 aa (dimer)	1000-1500 aa (monomer)
Guide RNA	ωRNA	crRNA and tracrRNA	crRNA and tracrRNA	crRNA
gRNA region	Located in protein ORF	Located in protein ORF	Exist alone	Exist alone
dsDNA target	5'TAM and target	5'PAM and target	5'PAM and target	5'PAM and target
PAM	5'-TTGAT / 5'-TCAN	5'-NAAN	5' T-rich PAM	5' T-rich PAM

张锋



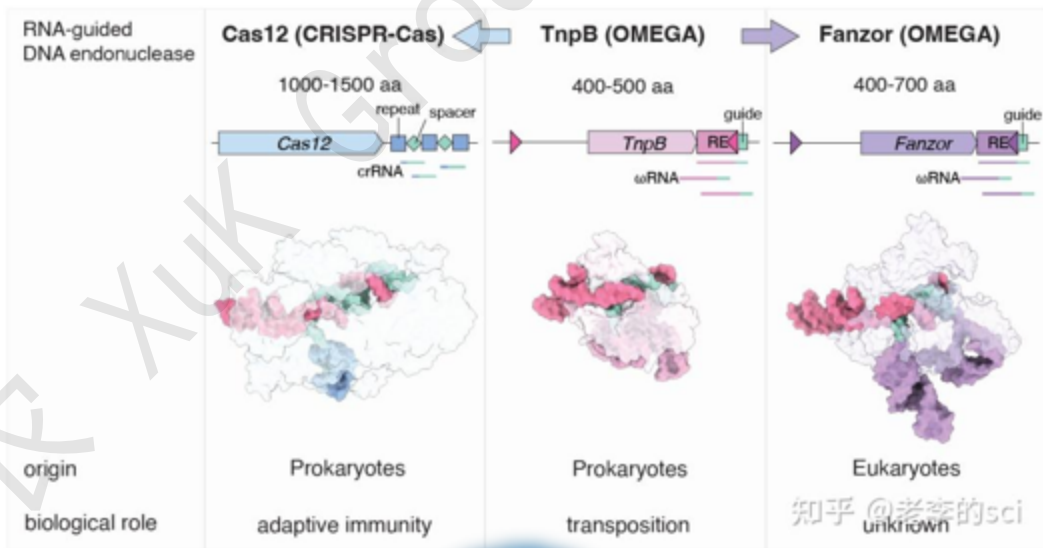
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“剪刀手”的进化



IscB → Cas9

TnpB → Cas12, Fanzor



张锋



**Cas9, Cas12, Cas13
IscB, TnpB, Fanzor
CRISPR筛选、检测等**



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知乎 @老李的sci

目录

- 01 十年磨一剑
- 02 昙花终一现
- 03 天选之骄子
- 04 黄粱成一梦
- 05 百花齐怒放
- 06 庄周梦有蝶

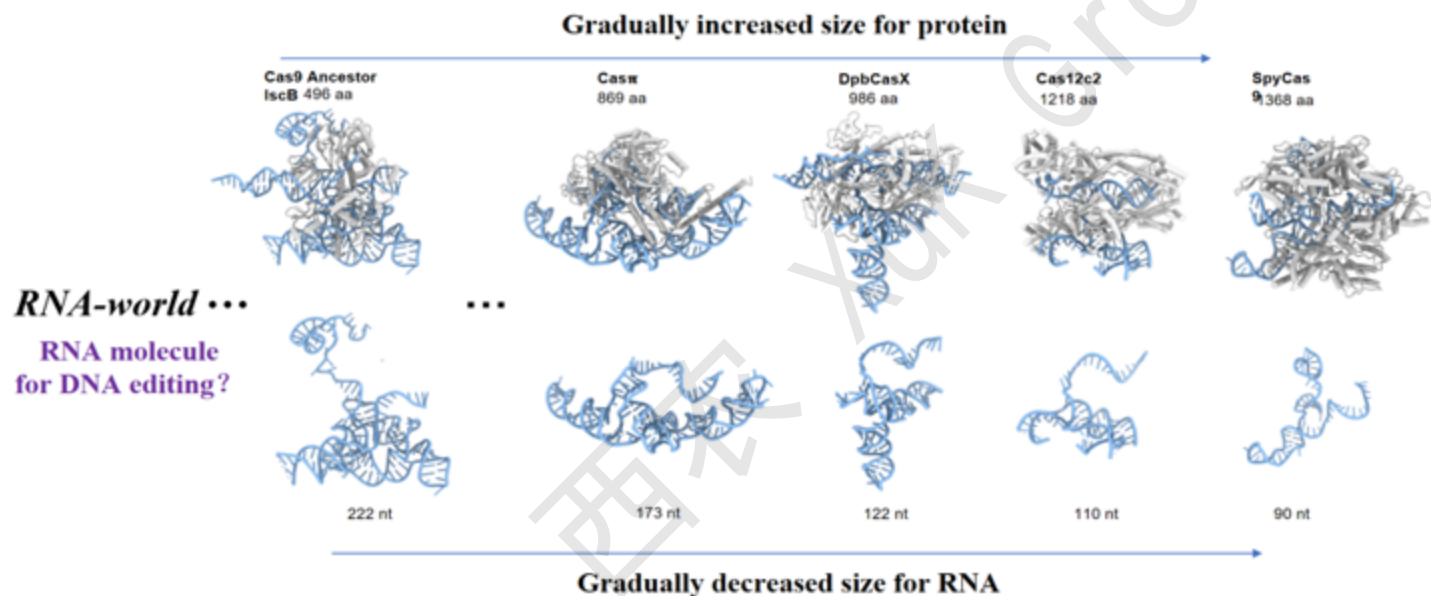


"RNA-World"

1. Background

RNA-Protein co-evolution starting from the RNA world

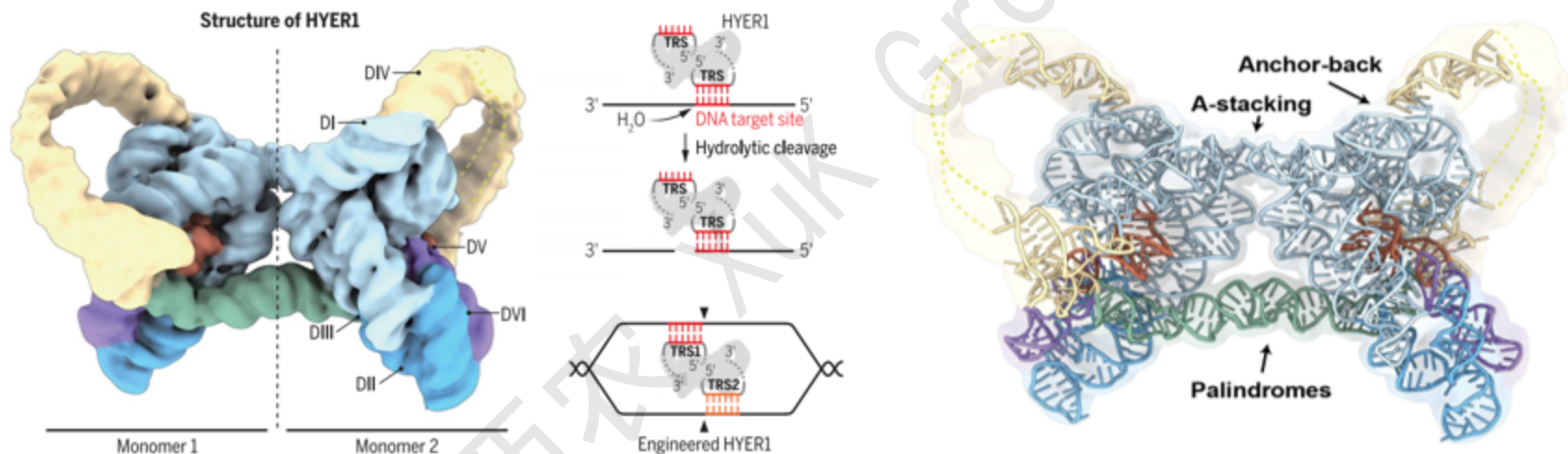
How is RNA gradually replaced by protein?



清华大学刘俊杰

Sun A. et al., *Cell Res*, 2023
Connor T.A. et al., *Mol. Cell*, 2022
Liu J.J. et al., *Nature*, 2019

纯RNA剪刀手:Hydrolytic endonucleolytic ribozyme (HYER)



<https://pubmed.ncbi.nlm.nih.gov/38301022/>

Liu Z, et al. *Science*. 2024.02

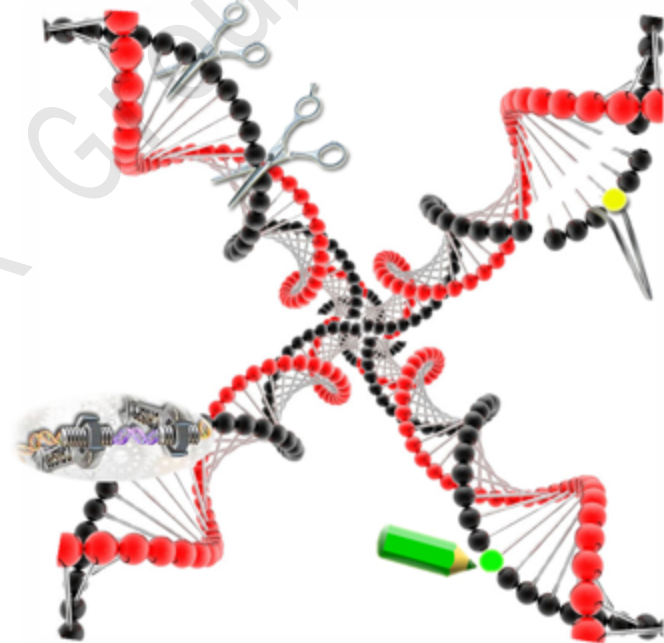


西北农林科技大学

拓展思考：

什么是基因编辑？

如何利用上述工具实现基因编辑？



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