巴基斯坦农业“千人计划”在华培训项目课程

基因编辑技术/Gene Editing Technology

基因编辑及衍生技术
Gene Editing and Beyond

徐坤/XU Kun　副教授/Associate professor

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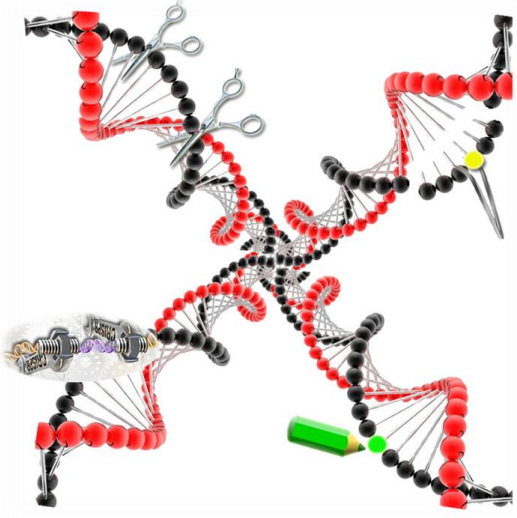
西北农林科技大学

Quick Review：

1. 画图+列表，对比总结归纳上述基因剪刀手; Draw diagrams and make tables, to compare and summarize the above-mentioned gene scissors

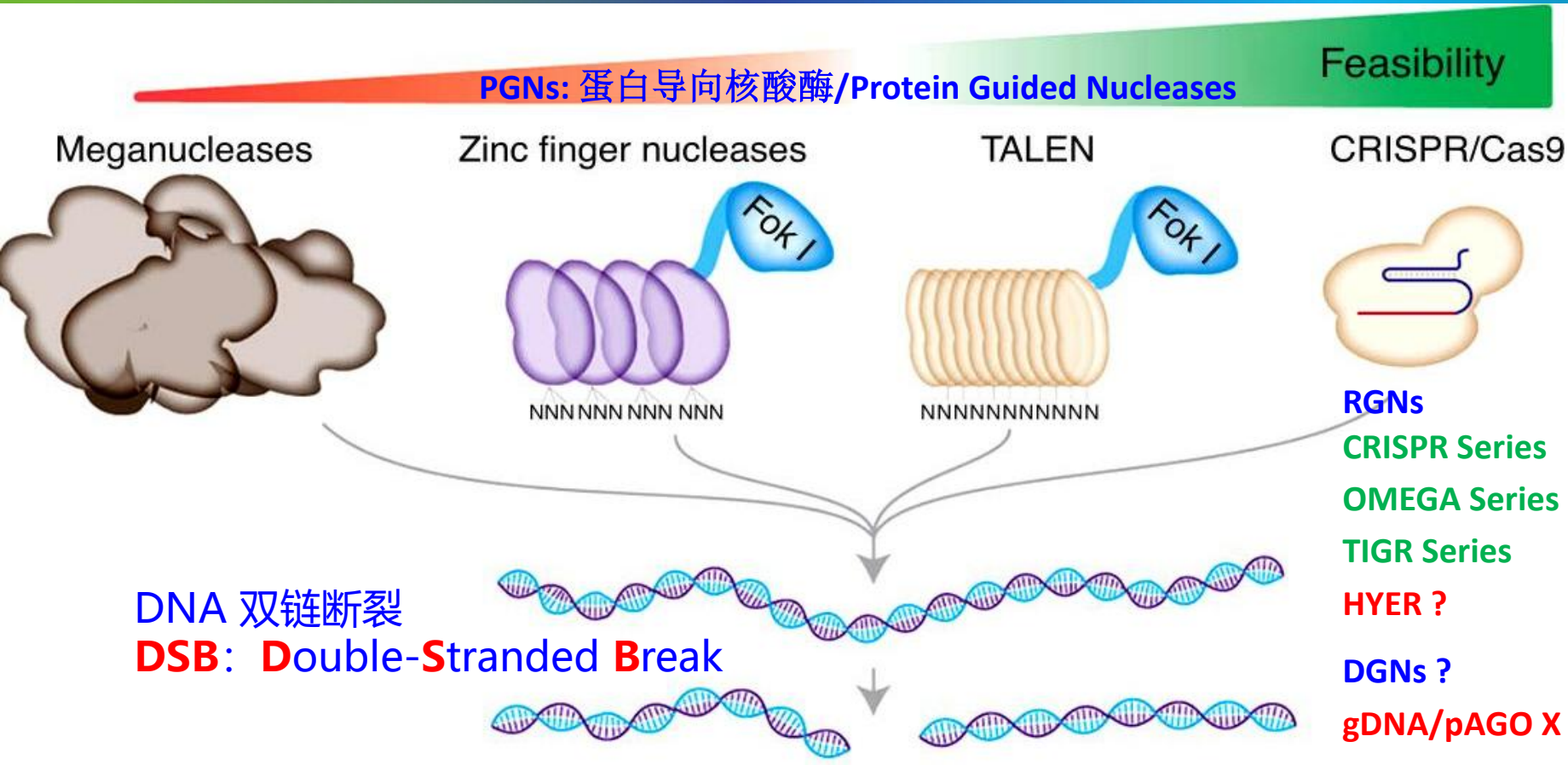
2. 思考如何利用上述工具实现基因编辑? Think about how to utilize the above-mentioned tools to achieve gene editing?



https://www.bilibili.com/video/BV15x411Z7kf?spm_id_from=333.788.videopod.episodes&vd_source=dac2eca651ed36d05d1ed333d1c7177d

基因剪刀手/Gene Scissors—

人工特异性核酸酶技术/Programmable specific endonuclease technology



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中国成语
Chinese idioms

Strategis for DSB repair

女娲：中国上古时代的神话人物
Mythological figures in ancient China

DNA双链断裂

DNA double-strand breaks

对于细胞来说

For cells

就是天塌下来的灾难！

It's a disaster like the sky falling!

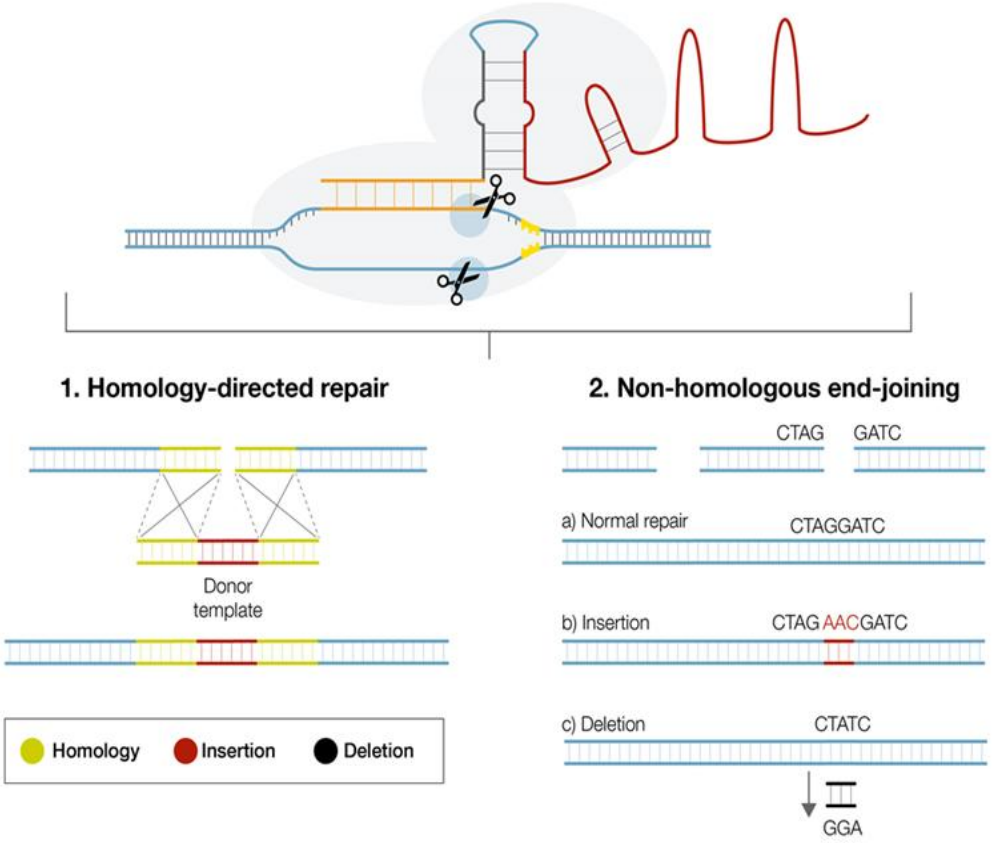
细胞的小宇宙

The microcosm of cells

也有自己的“女娲”

There is also her own "Nuwa"

补天之“石”/Stone for Mending the Sky—HDR & NHEJ



HDR:

Homology-Directed Repair

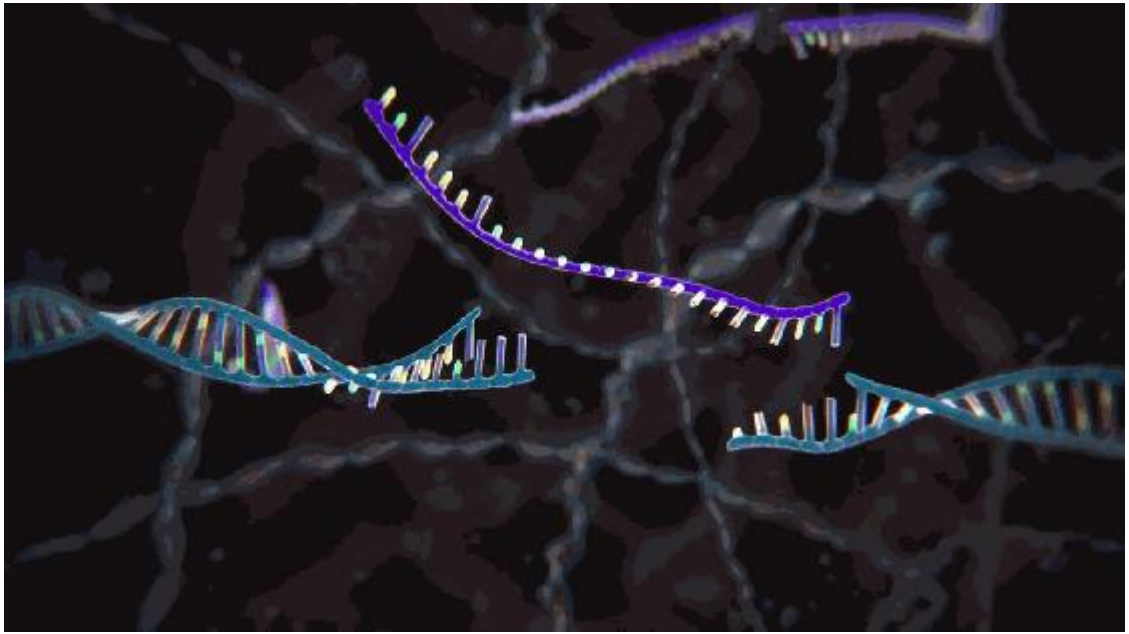
同源指导/引导/导向修复

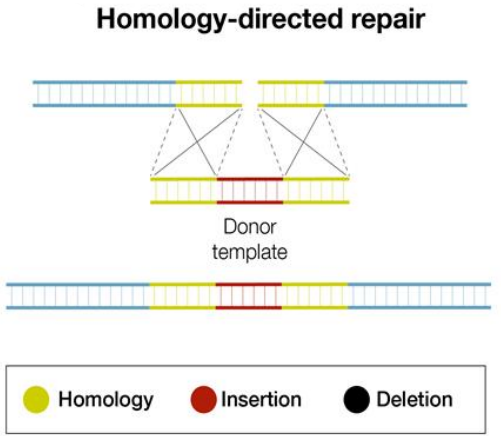
NHEJ:

Non-Homologous End-Joining

非同源末端连接

基于HDR的基因敲入（替换/点编辑）
Gene Knock-in (Replacing and Point editing) based on HDR

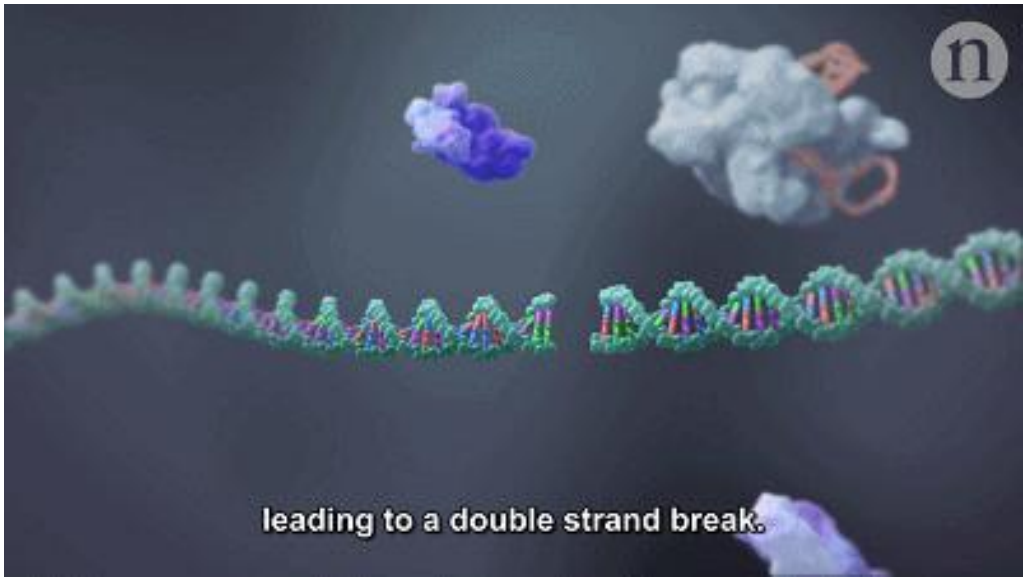


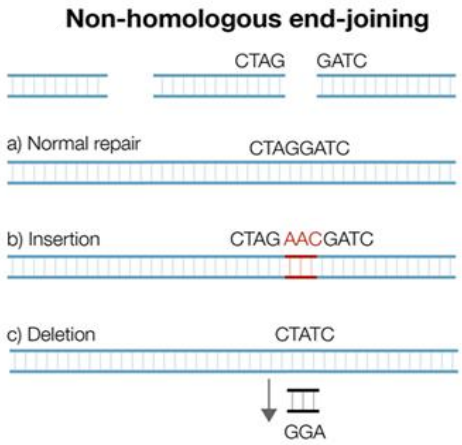


HDR→KI/Replace

HDR→Point/Base editing

利用NHEJ进行基因敲除
Gene Knock-out based on NHEJ





NHEJ→Indels→KO

NHEJ→Fragment deletion

NHEJ→插入或缺失（insertions and deletions, indels）→移码突变敲除/KO by Frameshift

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The Magic
Base Editor

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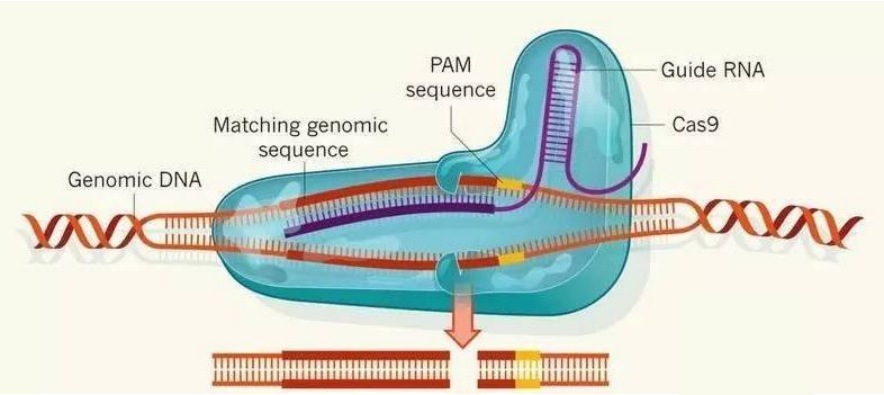
Ma Liang: character in a Chinese Fable

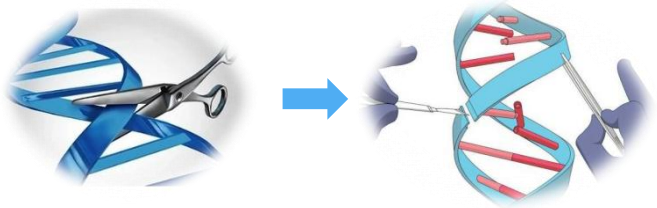
碱基编辑器/Base Editors

神笔马良的

Ma Liang's magic brush, which make the painting into reality

坏掉的剪刀/the Broken scissors—“nCas9”and“dCas9”





- **以CRISPR/Cas9为例, as an example**
- **Cas9缺口酶**（nicking Cas9, nCas9）：

  D10A **or** H840A
- **“死掉”的Cas9** (dead Cas9, dCas9):

  D10A **and** H840A

CBEs (Cytosine Base Editors) --based on Cytosine deaminase

Nature. 2016 May 19;533(7603):420-4. doi: 10.1038/nature17946. Epub 2016 Apr 20.

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Alexis C Komor [1,2], Yongjoo B Kim [1,2], Michael S Packer [1,2], John A Zuris [1,2], David R Liu [1,2]

Affiliations — collapse

Affiliations

1 Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, USA.
2 Howard Hughes Medical Institute, Harvard University, Cambridge, Massachusetts 02138, USA.

https://pubmed.ncbi.nlm.nih.gov/27096365/

Dveloped by David Liu,

BE1: **rAPOBEC1**-dCas9

BE2: rAPOBEC1-dCas9-**UGI**

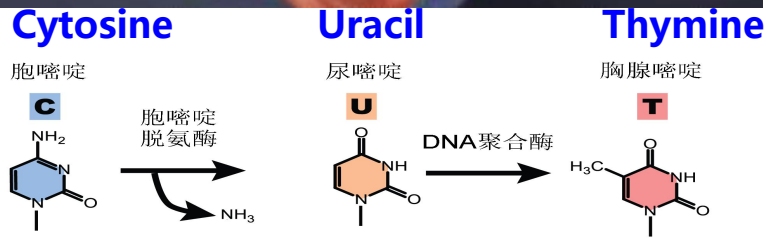
BE3: rAPOBEC1-**nCas9**-**UGI**

BE4: rAPOBEC1-**nCas9**-**2×UGI**

……

Uracil Glycosylase Inhibitor (UGI)

-NH_2: amidogen



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ABEs (Adenine Base Editors) --based on Adenine deaminase

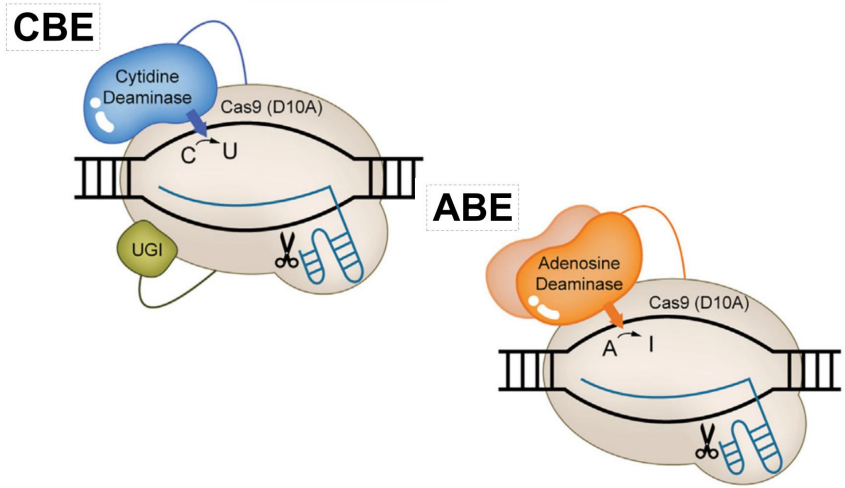
Comparative Study > Nature. 2017 Nov 23;551(7681):464-471. doi: 10.1038/nature24644.
Epub 2017 Oct 25.

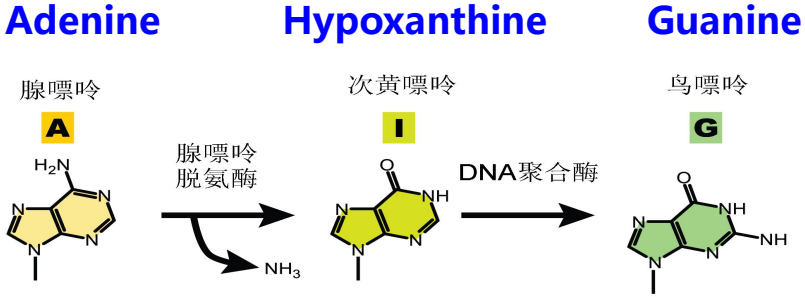
Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

Nicole M Gaudelli [1 2 3], Alexis C Komor [1 2 3], Holly A Rees [1 2 3], Michael S Packer [1 2 3], Ahmed H Badran [1 2 3], David I Bryson [1 2 3], David R Liu [1 2 3]

https://pubmed.ncbi.nlm.nih.gov/29160308/

ecTadA: *E. coli* tRNA adenosine deaminase





ABE7.10(ecTadA-ecTadA*-nCas9)

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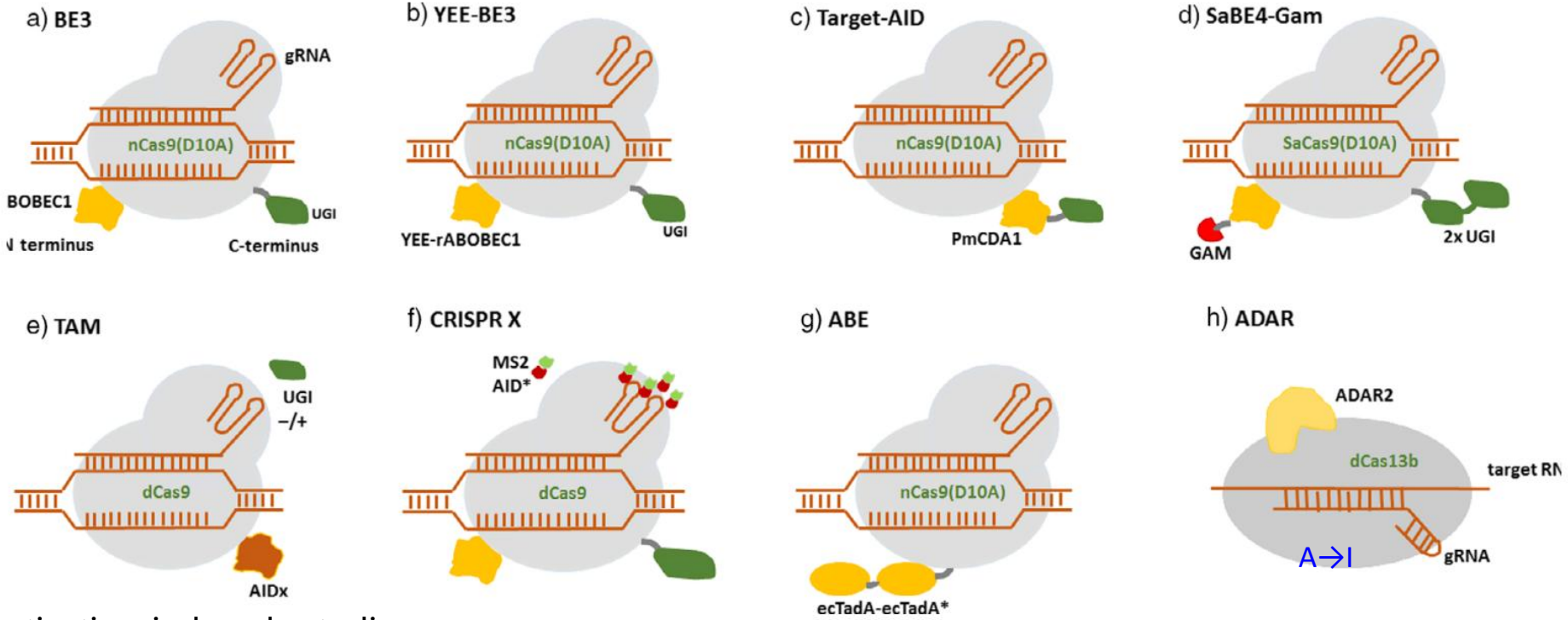
Different Versions of dBEs (deaminase-based Base Editors)



activation-induced cytodine deaminase (AID) ortholog **PmCDA1**

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https://pubmed.ncbi.nlm.nih.gov/31365173/

Different Versions of dBEs (deaminase-based Base Editors)

Engineered CRISPR–Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and **base editing**

BP Kleinstiver, AA Sousa, RT Walton, YE Tak… - Nature …, 2019 - nature.com

… enAsCas12a improves the efficiency of multiplex gene **editing**, endogenous gene activation and C-to-T **base editing**, and we engineered a high-fidelity version of enAsCas12a (…

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Hypercompact adenine **base editors** based on a Cas12f variant guided by engineered RNA

DY Kim, Y Chung, Y Lee, D Jeong, KH Park… - Nature chemical …, 2022 - nature.com

… Cas12f is a hypercompact type V, **Cas12** family member. Previously, we reported … **Editing** Technology). Having this feature in mind, we established TaRGET-based adenine **base editors** …

☆ Save 99 Cite Cited by 35 Related articles All 3 versions

[HTML] Engineering soybean with high levels of herbicide resistance with a Cas12-SF01-based cytosine **base editor**

Q Niu, H Xie, X Cao, M Song, X Wang… - Plant Biotechnology …, 2024 - pmc.ncbi.nlm.nih.gov

… We introduced the **base editor** into the elite soybean cultivar 'Xudou 18' through … The **base-editing** events at the crRNA target sites showed an overall 2.16% **editing** efficacy (with 9216 …

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Cas12i

Innate programmable DNA binding by CRISPR-Cas12m effectors enable efficient **base editing**

G Bigelyte, B Duchovska, R Zedaveinyte… - Nucleic Acids …, 2024 - academic.oup.com

… Cloning of **Cas12 base editors** expression vectors To obtain eukaryotic GoABE expression plasmid (pTK225), enAsCas12a sequence in enAsABE encoding plasmid (pTK221; gift from …

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Evolutionary mining and functional characterization of TnpB nucleases identify efficient miniature genome editors

G Xiang, Y Li, J Sun, Y Huo, S Cao, Y Cao, Y Guo… - Nature …, 2024 - nature.com

… Various domains have been fused to dCas or nCas, establishing epigenome editing, base editing and prime editing technologies 5,6 . However, due to the large size (>1,200 amino …

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Development of miniature base editors using engineered IscB nickase

D Han, Q Xiao, Y Wang, H Zhang, X Dong, G Li… - Nature …, 2023 - nature.com

… and its corresponding ωRNA to develop an IscB system that is highly efficient in … IscB-derived base editors (miBEs), exhibiting robust editing efficiency (up to 92%) to induce DNA base …

☆ 保存 99 引用 被引用次数：33 相关文章 所有 6 个版本

Engineered IscB–ωRNA system with expanded target range for base editing

Q Xiao, G Li, D Han, H Wang, M Yao, T Ma… - Nature Chemical …, 2024 - nature.com

… m16 RESH) and enωRNA) with robust editing activity and expanded the TAM range to … IscB.m16*-based adenine and cytosine base editors demonstrating robust base-editing efficiency …

☆ 保存 99 引用 相关文章 所有 3 个版本

Engineering miniature IscB nickase for robust base editing with broad targeting range

L Han, Y Hu, Q Mo, H Yang, F Gu, F Bai, Y Sun… - Nature Chemical …, 2024 - nature.com

… , it is critical to improve the editing efficiency. Here, we focused on … the editing efficiencies of IscB-mediated base editors. To test the editing efficiency of the IscB-mediated base editor, …

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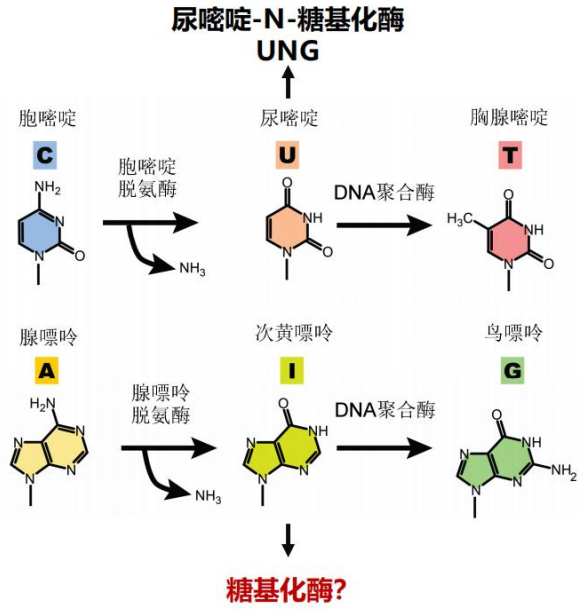
Mechanism of fighting against dBEs in cells

Uracil-DNA Glycosylase (UNG/UDG)



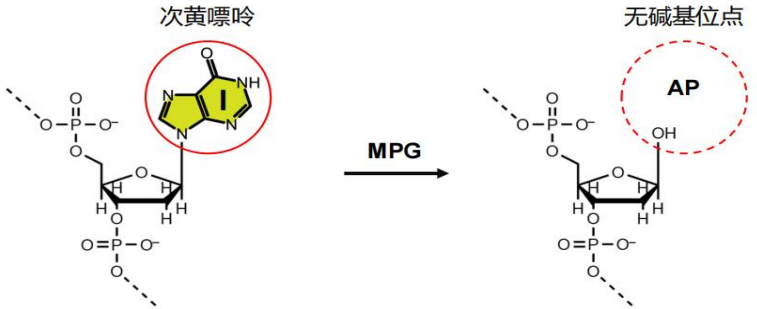
methylpurine DNA glycosylase (MPG)

MPG recognize and cut Hypoxanthine

N-甲基嘌呤 DNA 糖基化酶（MPG）具有切除次黄嘌呤的活性

人类 11 种 DNA 糖基化酶:

MPG, OGG1, MBD4, TDG, UNG, SMUG1, MUTYH, NTHL1, NEIL1, NEIL2, NEIL3



Abbrev.: **MPG**, N-methylpurine DNA Glycosylase; **I**, deoxyinosine; **AP**, Apurinic/apyrimidinic site

AP site: abasic site without any nucleoside

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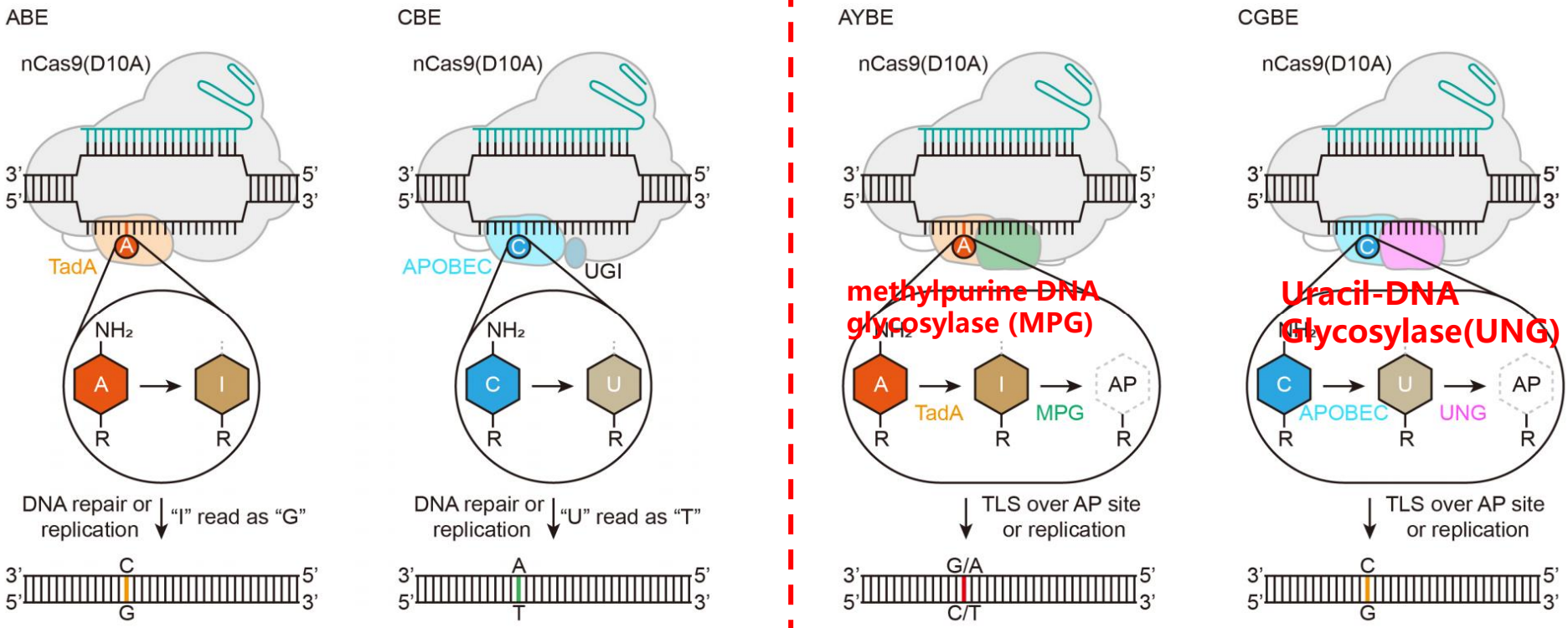
dBEs vs dgBEs (deaminase & glycosylase-based Base Editors)



dBEs：deaminase-based Base Editors

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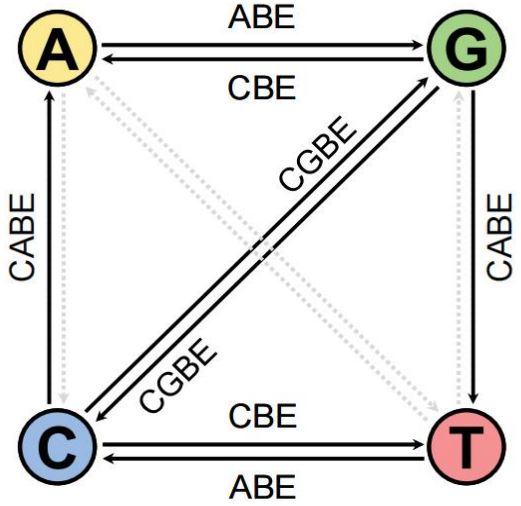
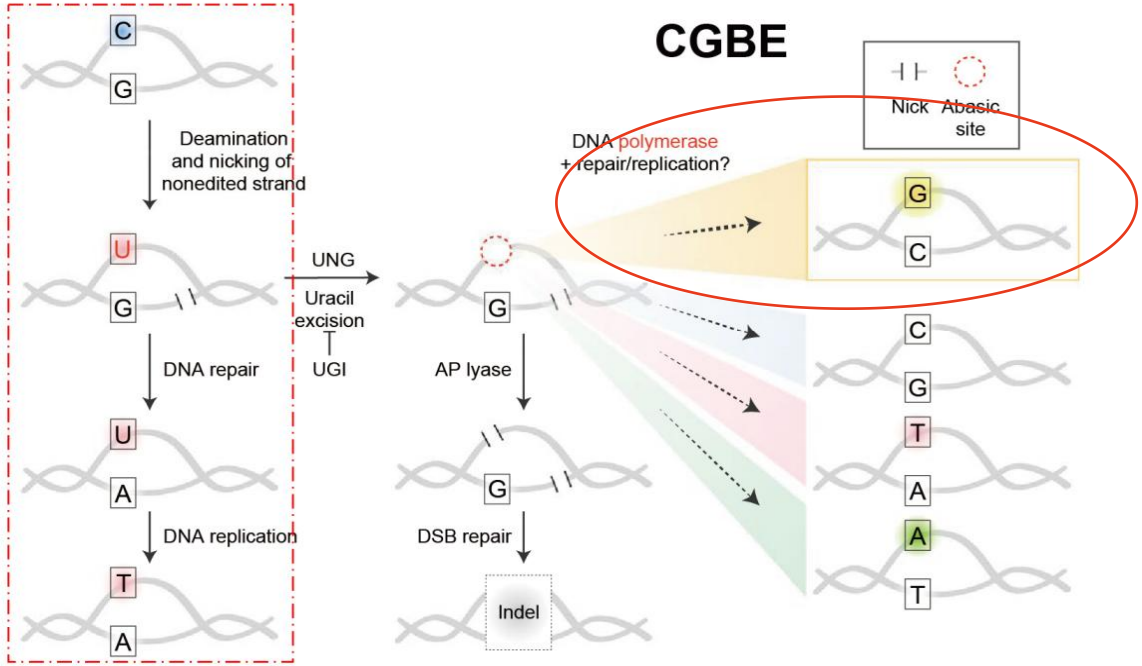
dgBEs

dgBEs--C-to-G Base editors (CGBE)





Abbrev.: **U**, Uracil; **UNG**, Uracil DNA Glycosylase; **AP**, Apurinic/apyrimidinic site; **DSB**, Double-Strand Break; **TLS**, Translesion Synthesis.

UNG=UDG

dgBEs--A-to-Y(C/T) Base editors (AYBE)

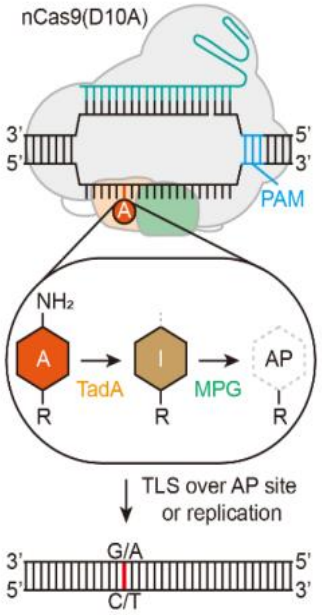
Nat Biotechnol. 2023 Aug;41(8):1080-1084. doi: 10.1038/s41587-022-01595-6. Epub 2023 Jan 9.

Programmable A-to-Y base editing by fusing an adenine base editor with an N-methylpurine DNA glycosylase

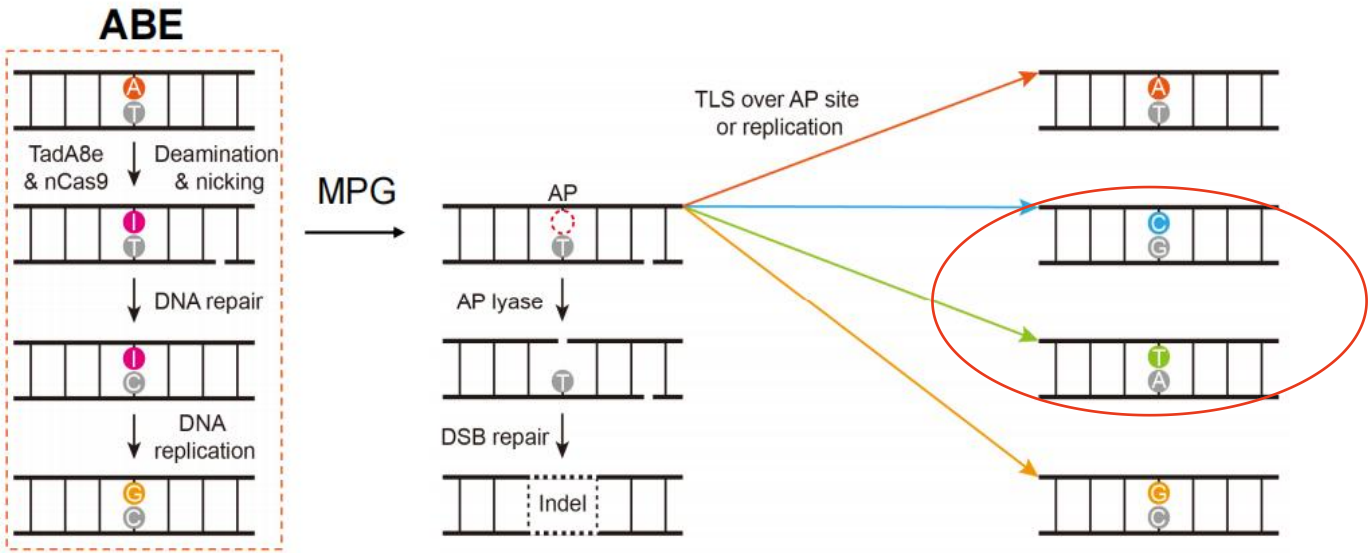
Huawei Tong [#1], Xuchen Wang [#2,3], Yuanhua Liu [#2], Nana Liu [#4], Yun Li [4], Jiamin Luo [4], Qian Ma [4], Danni Wu [4], Jiyong Li [4], Chunlong Xu [5], Hui Yang [6,7,8,9]

AYBE 原型设计

ABE-MPG 融合



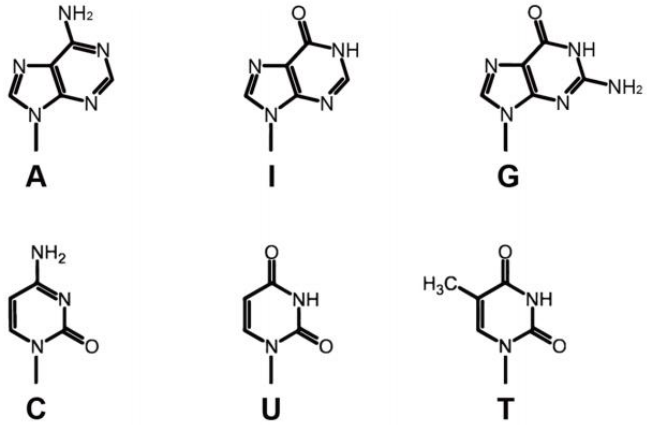
AYBE 碱基编辑原理

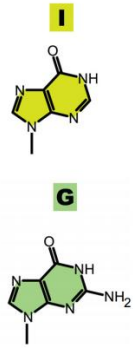


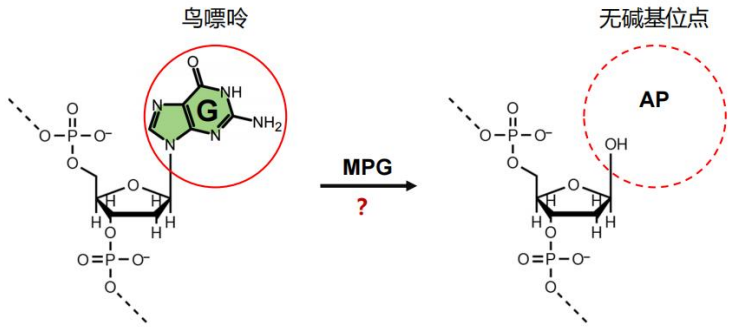
Abbrev.: **AYBE**, adenine transversion base editor; **MPG**, N-methylpurine DNA Glycosylase; **I**, deoxyinosine; **AP**, Apurinic/apyrimidinic site; **DSB**, Double-Strand Break; **TLS**, Translesion Synthesis.

https://pubmed.ncbi.nlm.nih.gov/36624150/

How about Guanine and Thymine?



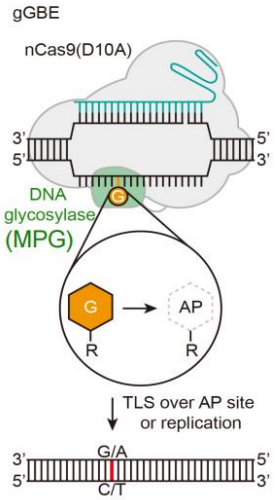




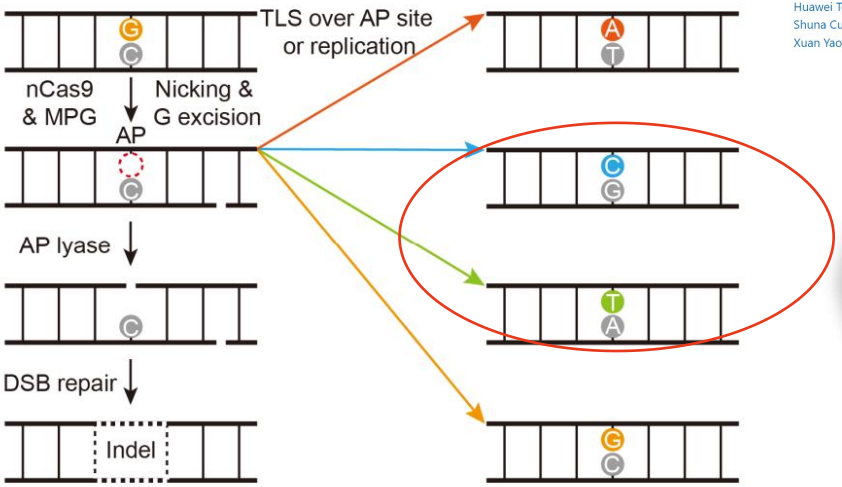
G and T cannot be converted into other bases through deamination reactions

gBEs（glycosylase-based Base Editors）--gGBE（GYBE）

gGBE 原型设计



gGBE 碱基编辑原理



Abbrev.: **gGBE**, glycosylase-based guanine base editor; **MPG**, N-methylpurine DNA Glycosylase; **I**, deoxyinosine; **AP**, Apurinic/apyrimidinic site; **DSB**, Double-Strand Break; **TLS**, Translesion Synthesis.

Natl Sci Rev. 2023 May 16;10(8):nwad143. doi: 10.1093/nsr/nwad143. eCollection 2023 Aug.

Programmable deaminase-free base editors for G-to-Y conversion by engineered glycosylase

Huawei Tong [1], Nana Liu [1], Yinghui Wei [1], Yingsi Zhou [1], Yun Li [1], Danni Wu [1], Ming Jin [2], Shuna Cui [1], Hengbin Li [1], Guoling Li [1], Jingxing Zhou [1], Yuan Yuan [1], Hainan Zhang [1], Linyu Shi [1], Xuan Yao [1], Hui Yang [1 3 4]

杨辉

中科院药物所 童华威

西北农林科技大学

https://pubmed.ncbi.nlm.nih.gov/37404457/

gBEs (glycosylase-based Base Editors) --gCBE & gTBE

Nat Biotechnol. 2024 Oct;42(10):1538-1547. doi: 10.1038/s41587-023-02050-w. Epub 2024 Jan 2.

Glycosylase-based base editors for efficient T-to-G and C-to-G editing in mammalian cells

Lijun Ye [#1,2,3], Dongdong Zhao [#1,2,3], Ju Li [#4], Yiran Wang [1,3,4], Bo Li [1,2,3], Yuanzhao Yang [1,3,5], Xueting Hou [1,3], Huibin Wang [1,3,5], Zhandong Wei [1,3], Xiaoqi Liu [1,3], Yaqiu Li [1,3], Siwei Li [1,3], Yajing Liu [1,3], Xueli Zhang [6,7,8], Changhao Bi [9,10,11]

Mol Cell. 2024 Apr 4;84(7):1257-1270.e6. doi: 10.1016/j.molcel.2024.01.021. Epub 2024 Feb 19.

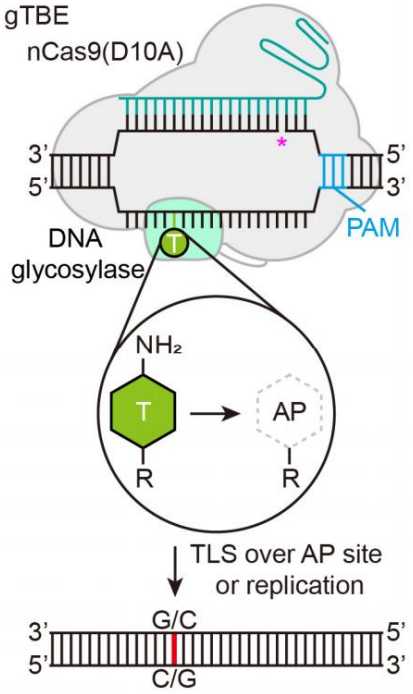
Protein language models-assisted optimization of a uracil-N-glycosylase variant enables programmable T-to-G and T-to-C base editing

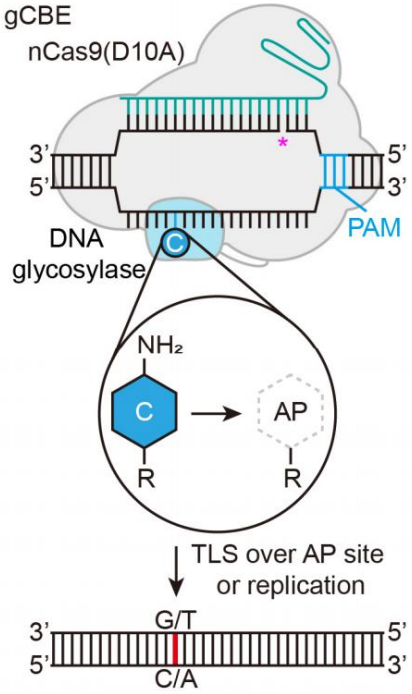
Yan He [1], Xibin Zhou [2], Chong Chang [3], Ge Chen [3], Weikuan Liu [1], Geng Li [3], Xiaoqi Fan [3], Mingsun Sun [3], Chensi Miao [3], Qianyue Huang [3], Yunqing Ma [3], Fajie Yuan [4], Xing Chang [5]

Nat Commun. 2024 Jun 8;15(1):4897. doi: 10.1038/s41467-024-49343-5.

Development of deaminase-free T-to-S base editor and C-to-G base editor by engineered human uracil DNA glycosylase

Huawei Tong [#1], Haoqiang Wang [#2], Xuchen Wang [#3,4], Nana Liu [#2], Guoling Li [2], Danni Wu [2], Yun Li [2], Ming Jin [5], Hengbin Li [2], Yinghui Wei [6,7], Tong Li [2], Yuan Yuan [2], Linyu Shi [2], Xuan Yao [2], Yingsi Zhou [8], Hui Yang [9,10]





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Partial Chinese Scientists Contributed to Base Editor Development

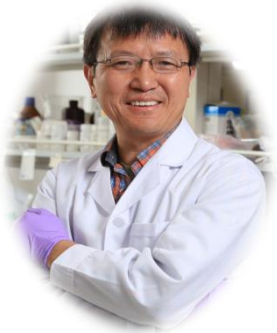
李大力/Li Dali

毕昌昊/Li Dali

高彩霞/Gao Caixia

魏文胜/Wei WenSheng

常兴/Chang Xing



黄行许/Huang Xingxu

杨辉/Yang Hui

陈佳/Chen Jia

汪阳明/Wang Yangming

童华威/Tong Huawei

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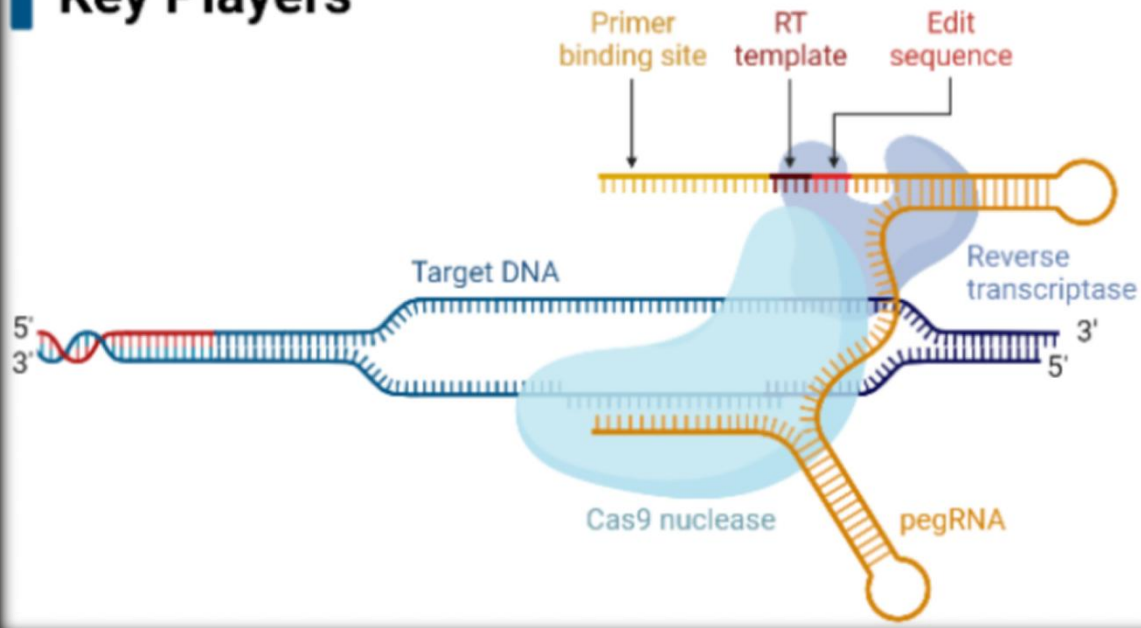
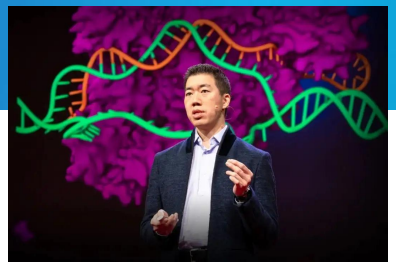
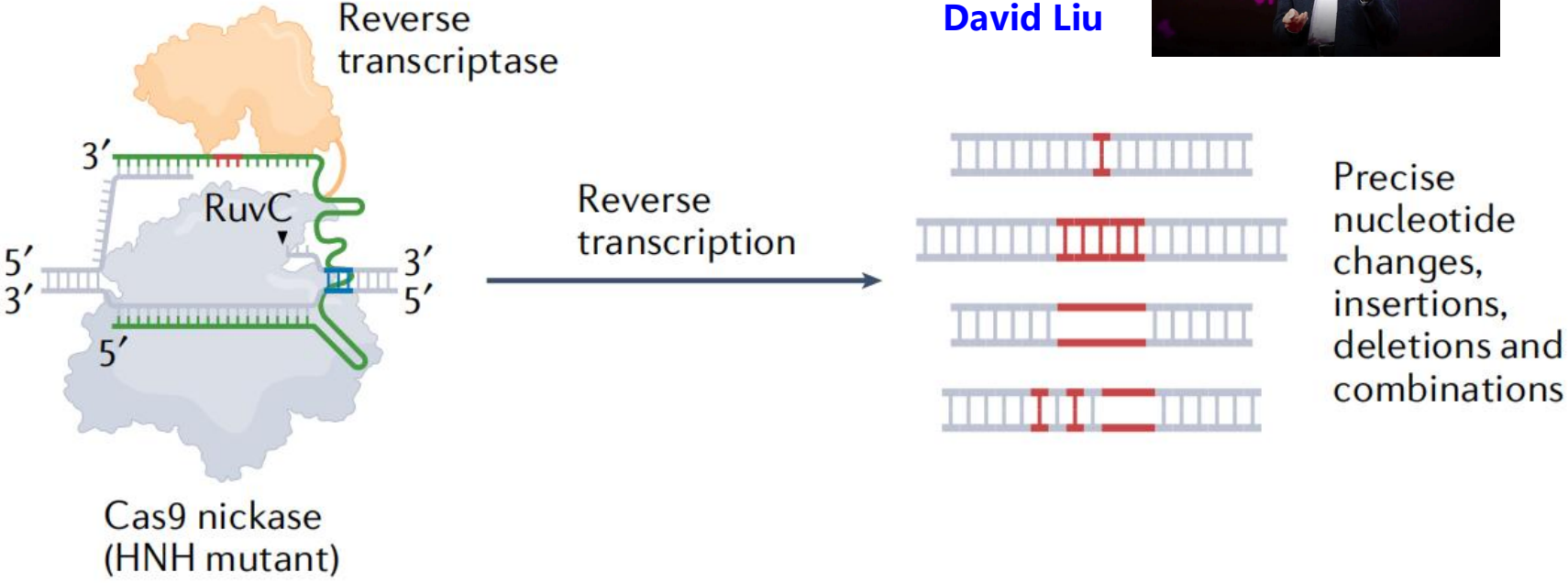
Prime Editing Key Players



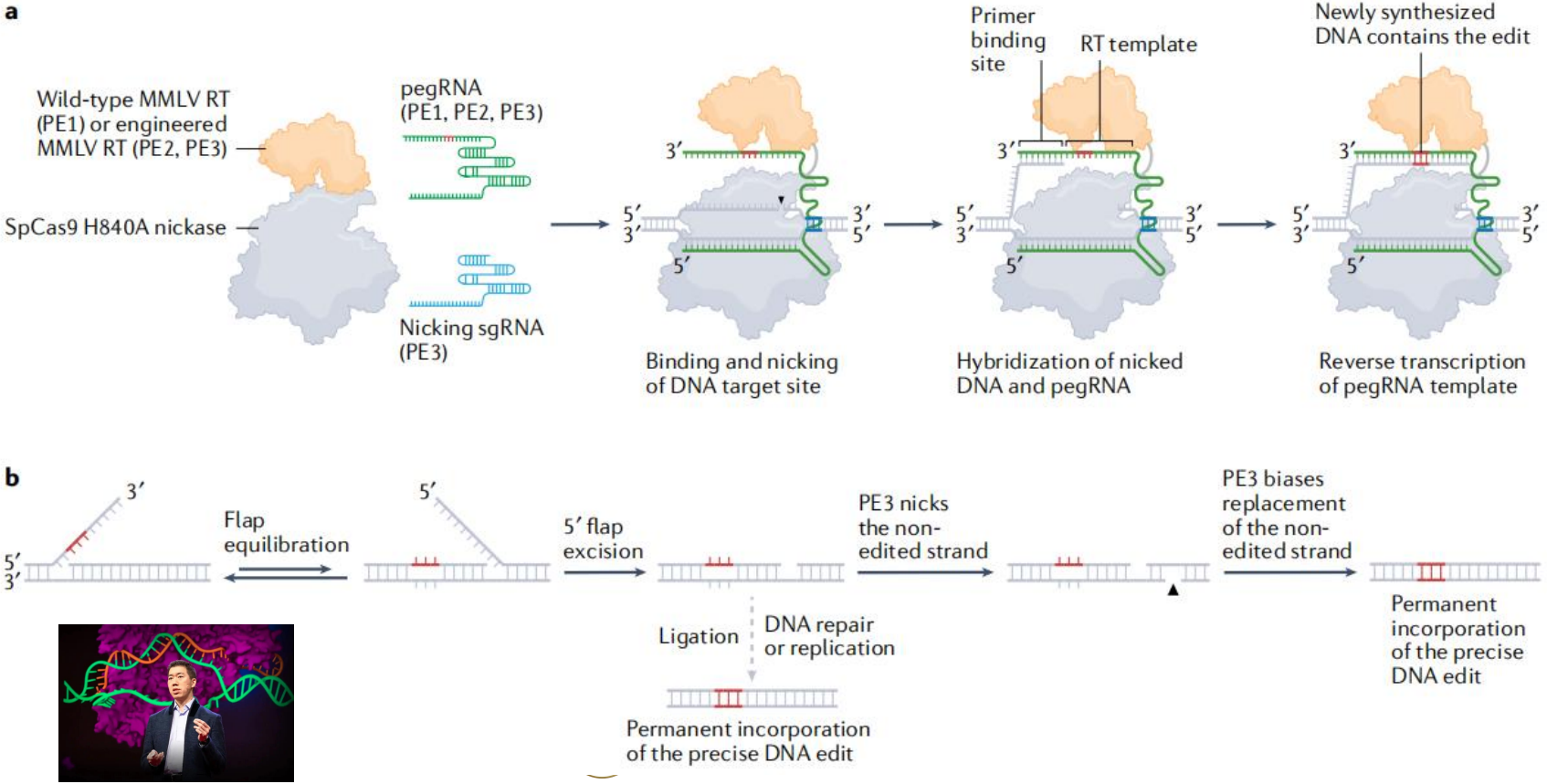
1. PE1 and beyond



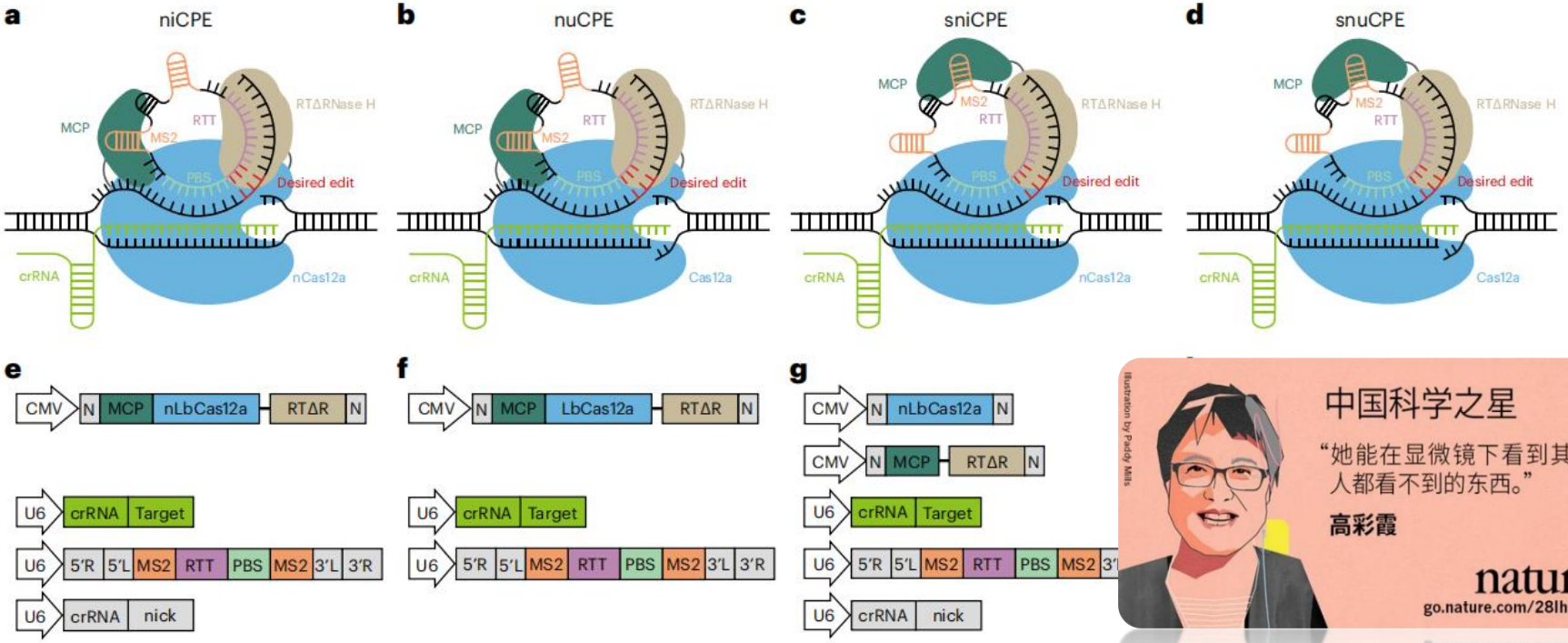
刘如谦
David Liu



2. PE2 and beyond



3. circular RNA-mediated PEs



Recruitment by MCP

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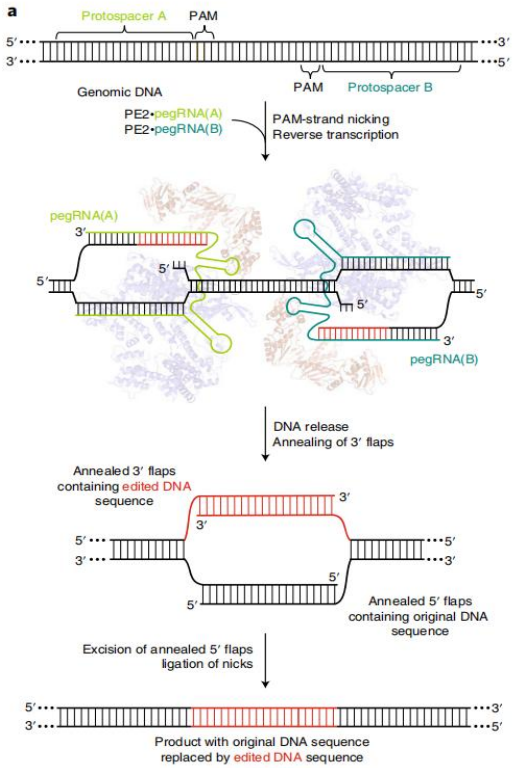
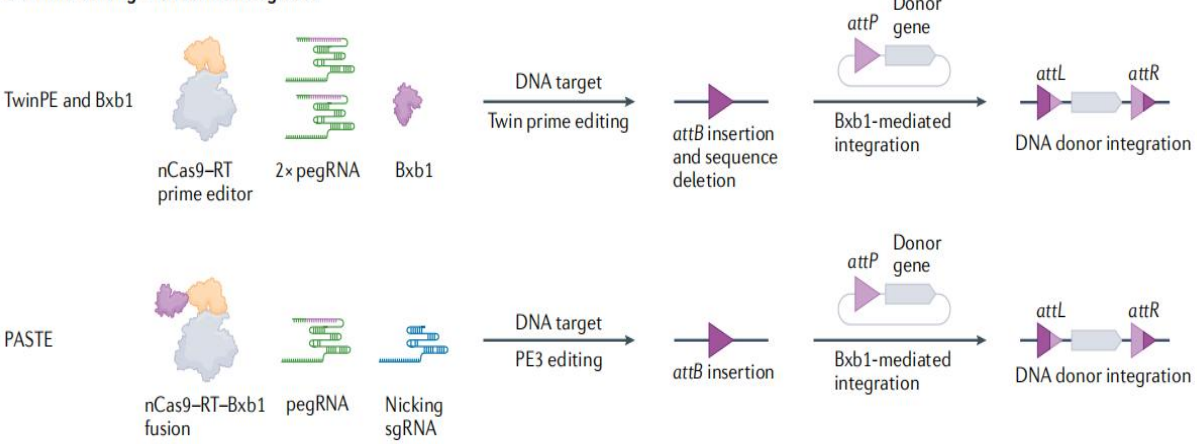
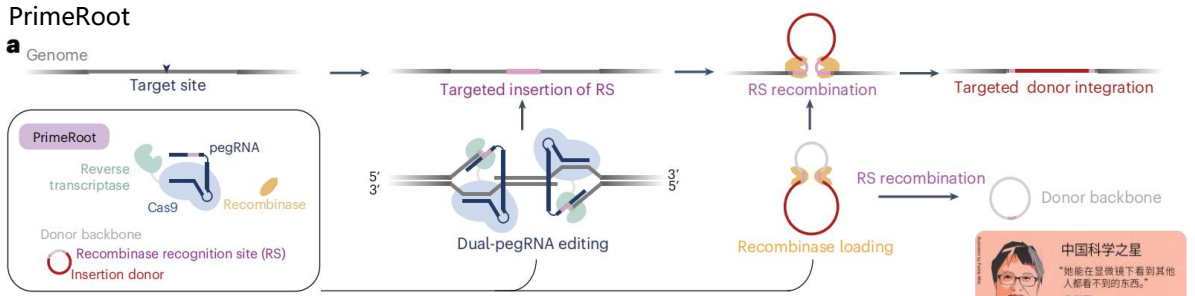
高彩霞/Gao Caixia

4. twinPE and beyond







5. Click editors

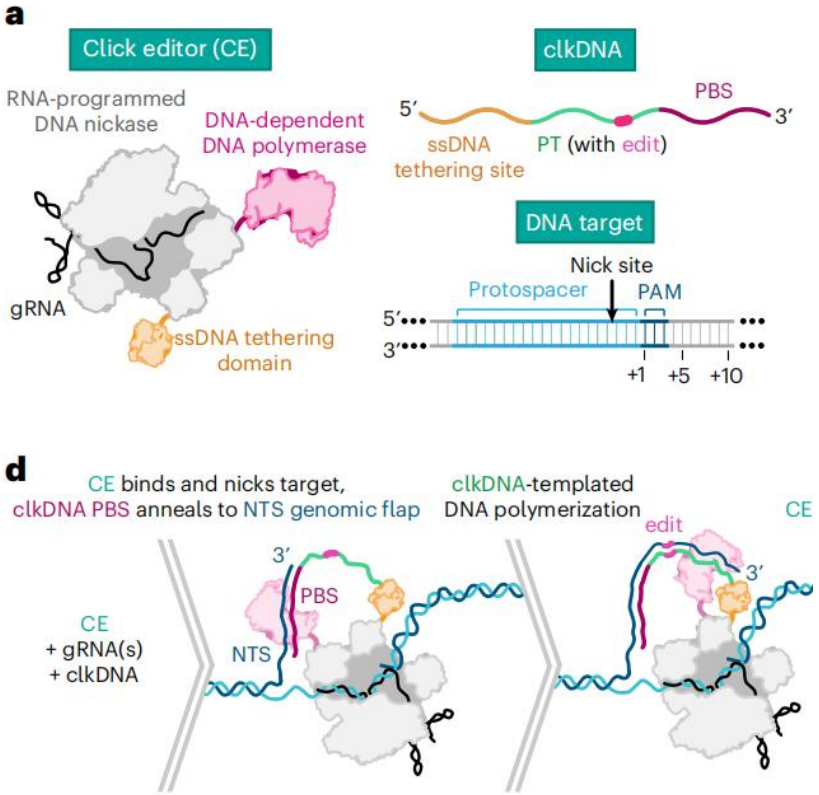
› Nat Biotechnol. 2024 Jul 22:10.1038/s41587-024-02324-x. doi: 10.1038/s41587-024-02324-x.
Online ahead of print.

Click editing enables programmable genome writing using DNA polymerases and HUH endonucleases

Joana Ferreira da Silva [#1 2 3], Connor J Tou [#1 2 4], Emily M King [1 2 5], Madeline L Eller [1 2], David Rufino-Ramos [1 2 3], Linyuan Ma [1 2 3], Christopher R Cromwell [1 2 3], Jasna Metovic [1 6 7], Friederike M C Benning [8 9], Luke H Chao [8 9], Florian S Eichler [1 6 7], Benjamin P Kleinstiver [10 11 12]

Affiliations + expand

PMID: 39039307 PMCID: PMC11751136 (available on 2026-01-22)
DOI: 10.1038/s41587-024-02324-x



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Amazing and
Versatile CRISPR

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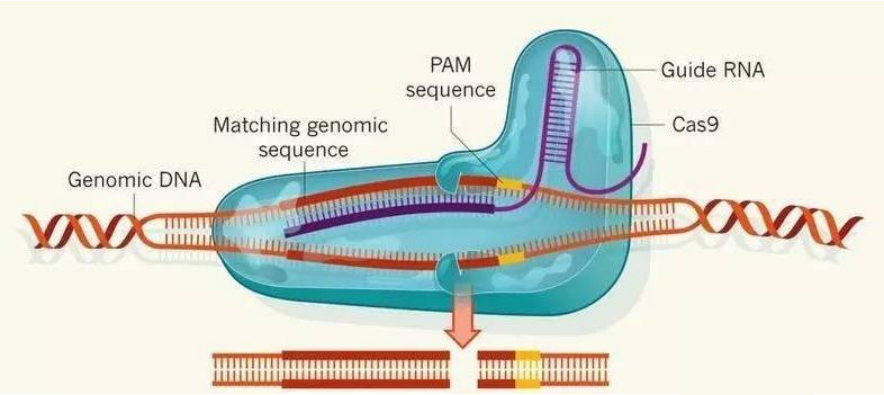
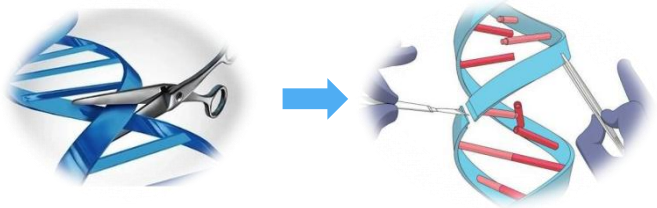
坏掉的剪刀/the Broken scissors—“nCas9”and“dCas9”





- **以CRISPR/Cas9为例, as an example**
- **Cas9缺口酶**（nicking Cas9, nCas9）：

  D10A **or** H840A
- **“死掉”的Cas9** (dead Cas9, dCas9):

  D10A **and** H840A

CRISPR之凤凰涅槃

凤凰：The auspicious beast second only to the dragon in Chinese mythology，which is assumed can reborn from the ashes

凤凰涅槃—dCas9

Phenix Rebirth - dCas9

Cas9死了？不，

Is Cas9 dead? No，

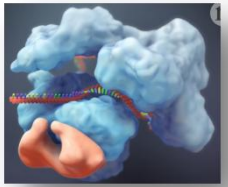
它还活着！

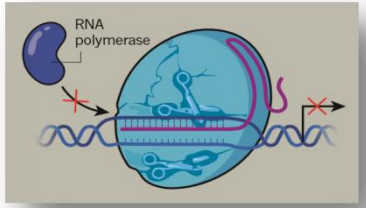
It is still alive!

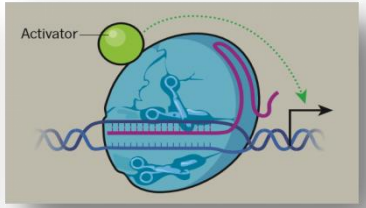
并且很精彩！

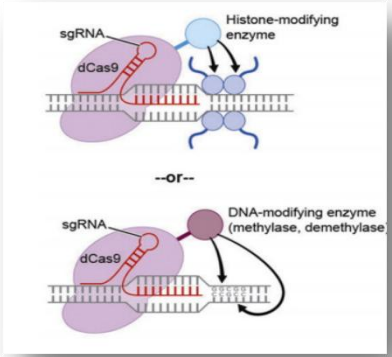
And a wonderful living!

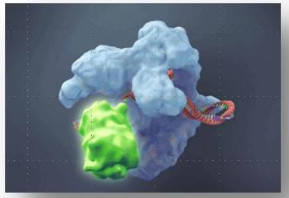
“八臂哪吒” /Nezha’s eight arms-- CRISPR-derived technologies

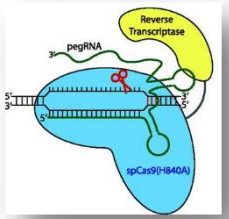


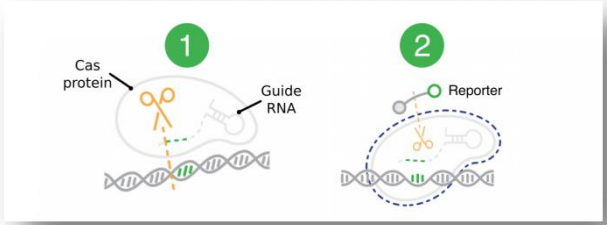












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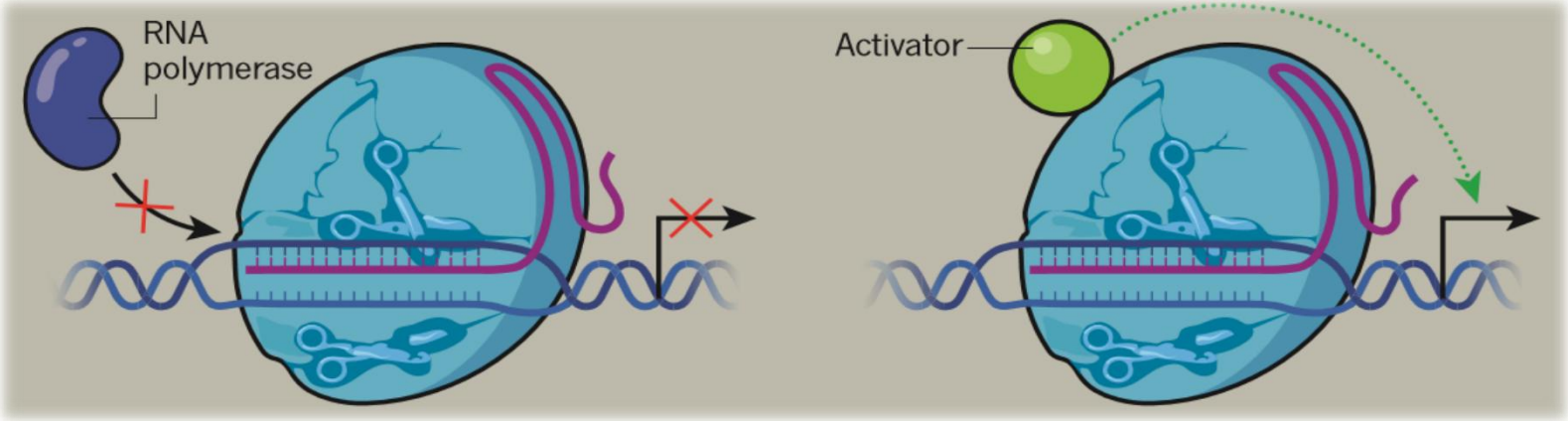
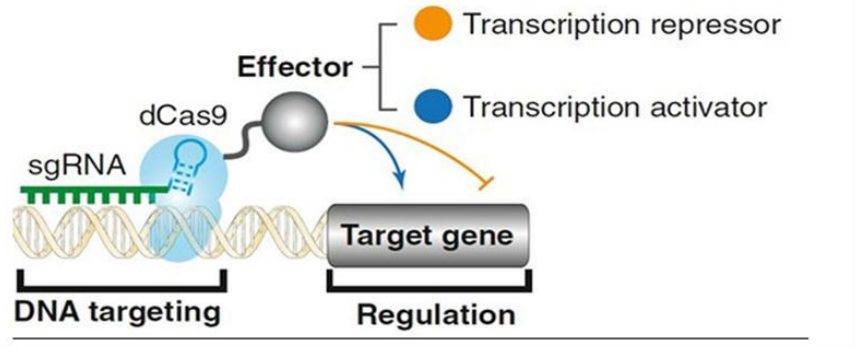
“八臂哪吒” /Nezha’s eight arms-- CRISPRi and CRISPRa



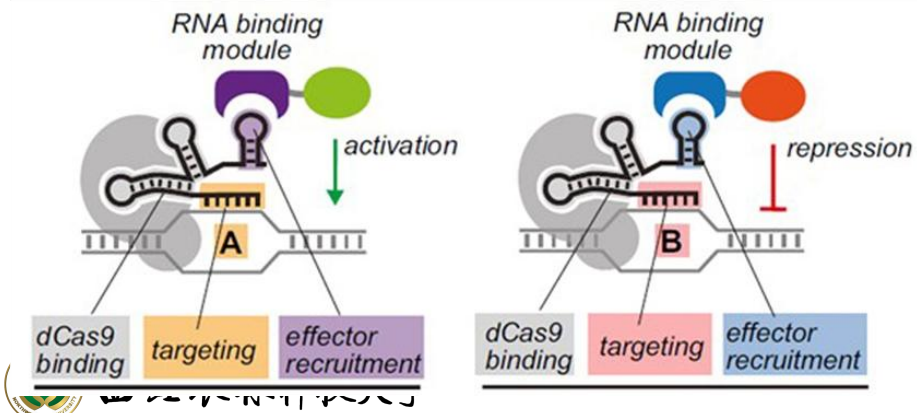
- CRISPR interference（CRISPRi）
- CRISPR activator（CRISPRa）

“八臂哪吒”/Nezha’s eight arms-- CRISPR transcription factors

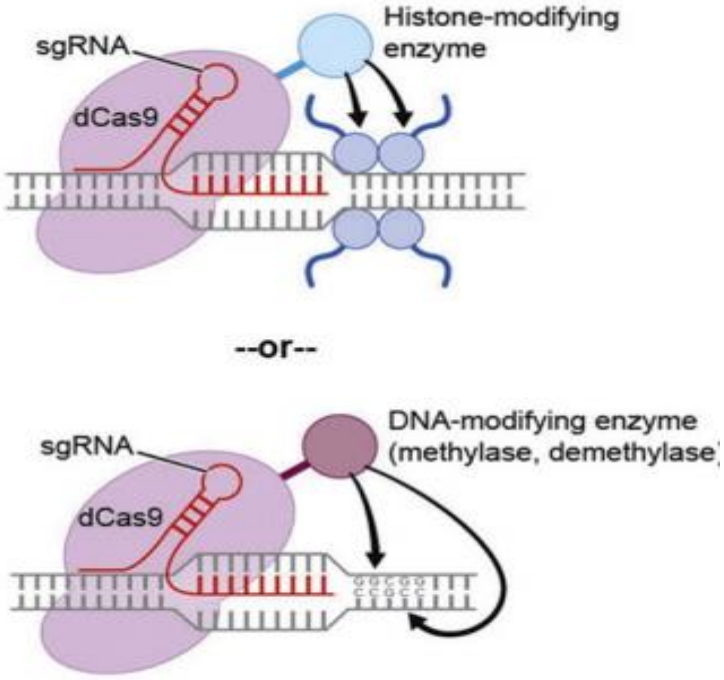
(1) dCas9 fusion



(2) sgRNA tether

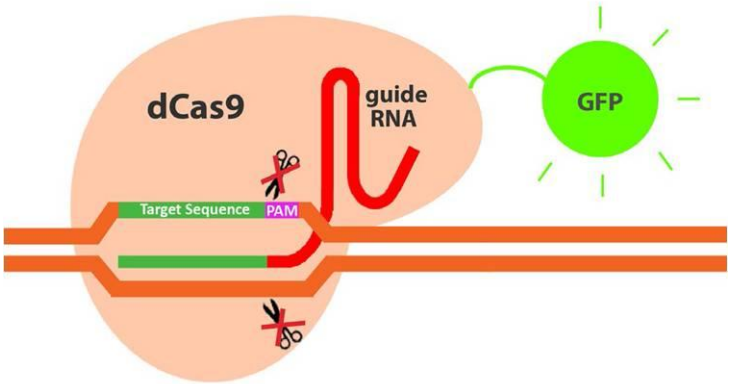


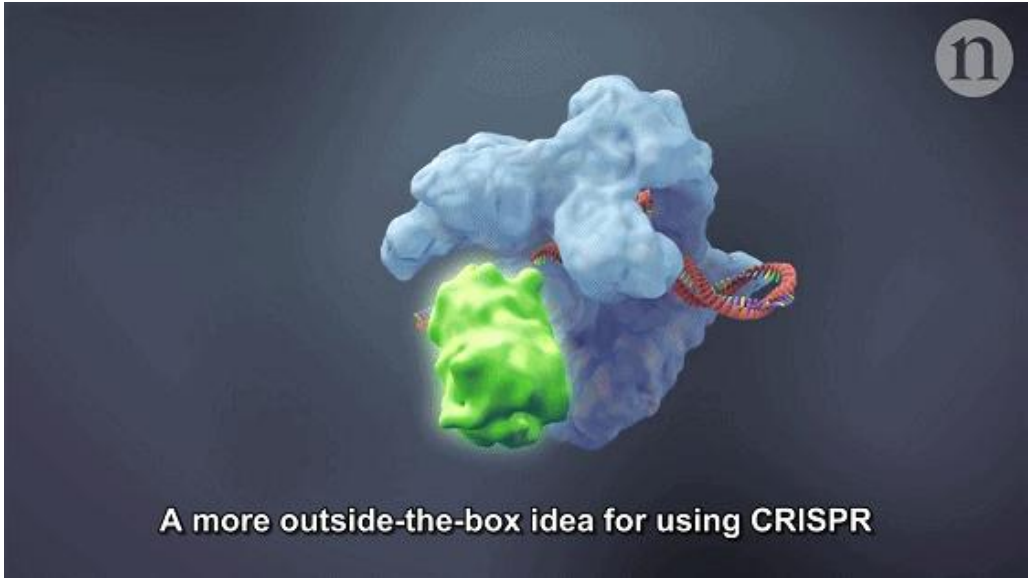
“八臂哪吒” /Nezha’s eight arms-- CRISPR epigenetic regulation factors



dCas9 fusion/sgRNA tether

“八臂哪吒” /Nezha’s eight arms-- CRISPR imaging

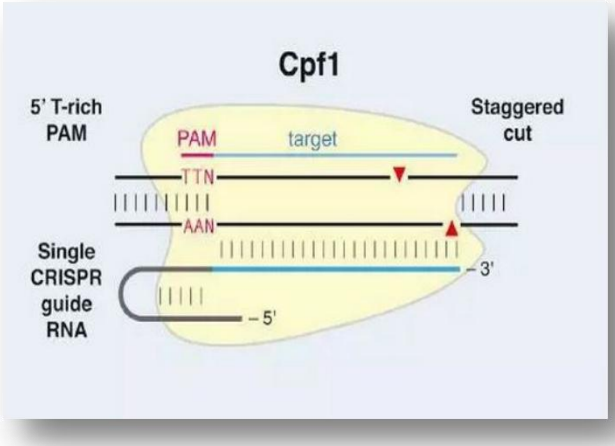


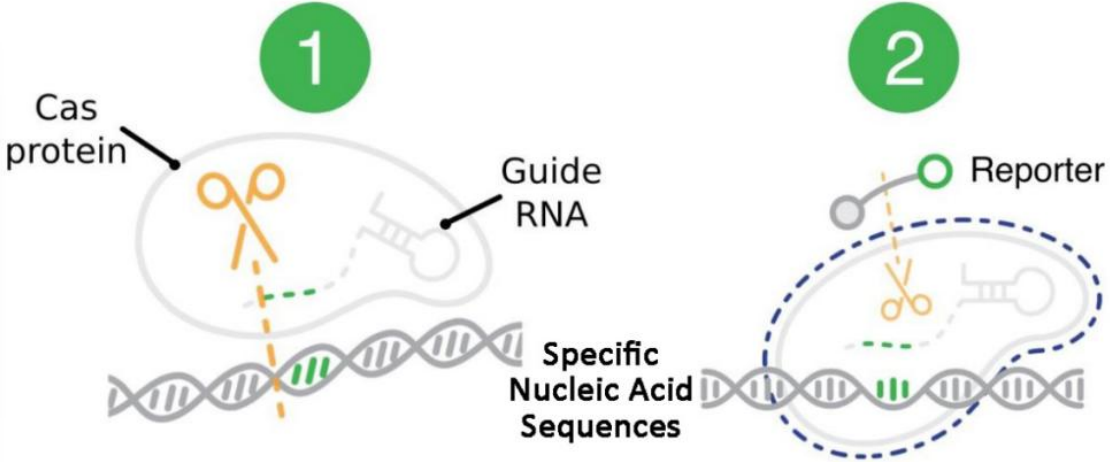


Genomic dynamic imaging and living cell tracing

“八臂哪吒” /Nezha’s eight arms-- CRISPR detetion

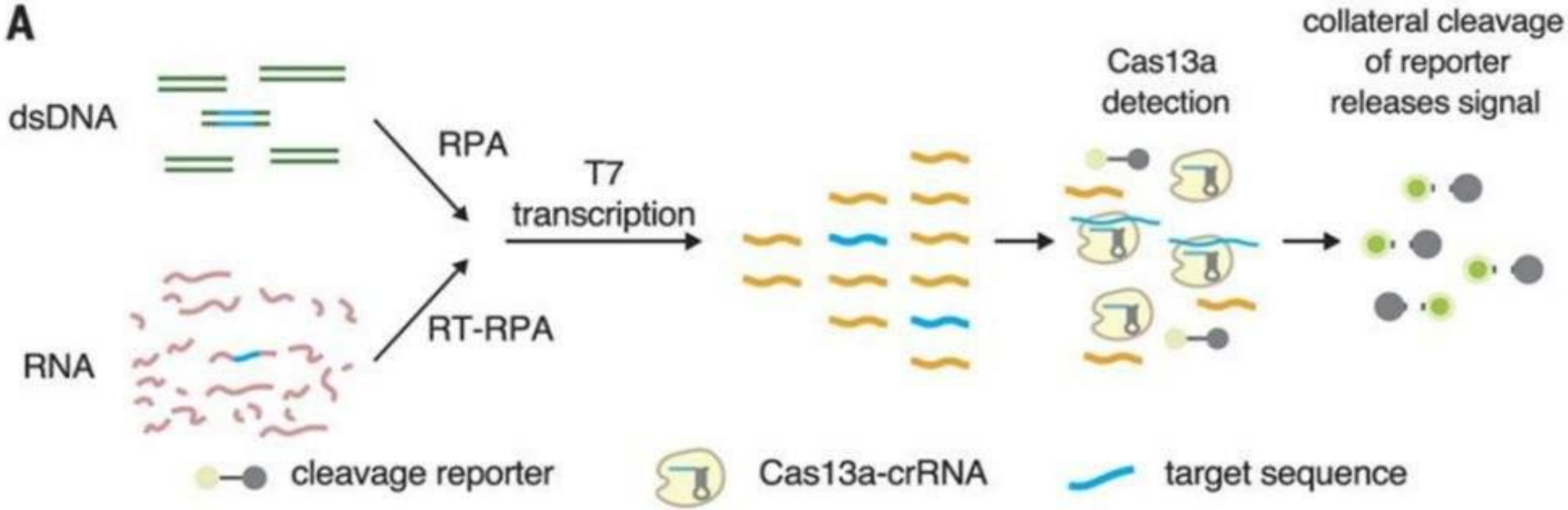
DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR)





CRISPR/Cas12a Non-specific bypass-cutting effect

Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK)



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Infinite possible by gene editing and beyond technologies

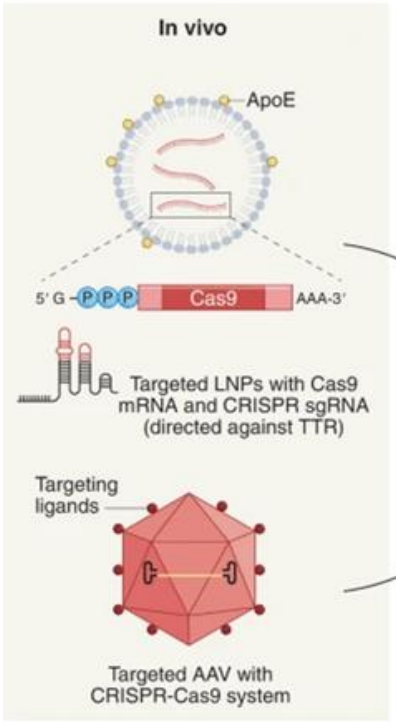
西北农林科技大学

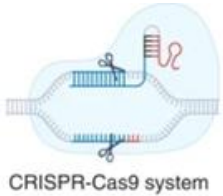
Gene editing and beyond —

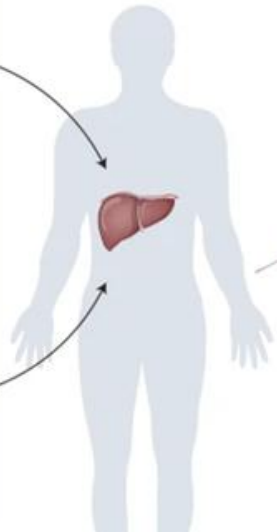
改变世界的技术/Technologies that changing the world

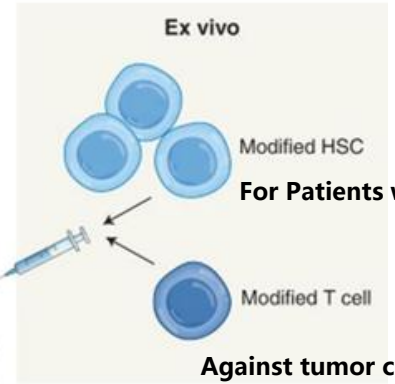
Engineering of **Animal**, **Plant** and **Microbe** lifes for **Agricultural Breeding**, **Gene Therapy**, as well as **Life Investigation**.

In vivo & Ex vivo Gene Therapy



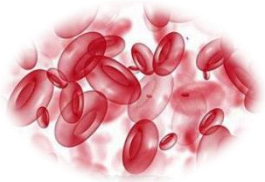




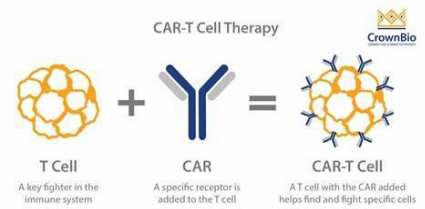


Against tumor cells

Hematopoietic stem cells (HSC) for differentiating into various blood cells



For Patients with anemia



Targeting viruses lurking in the human body

CLON 中科奥格 RGAN

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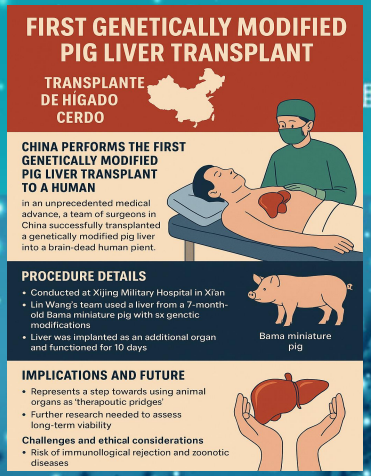
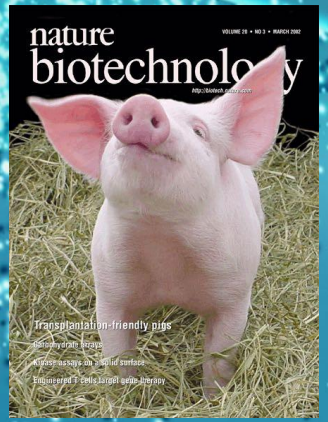
Building the organ factory , xenotransplantation changes the future

潘登科博士
Dr. Pan DK

打造未来器官工厂 异种移植改变未来

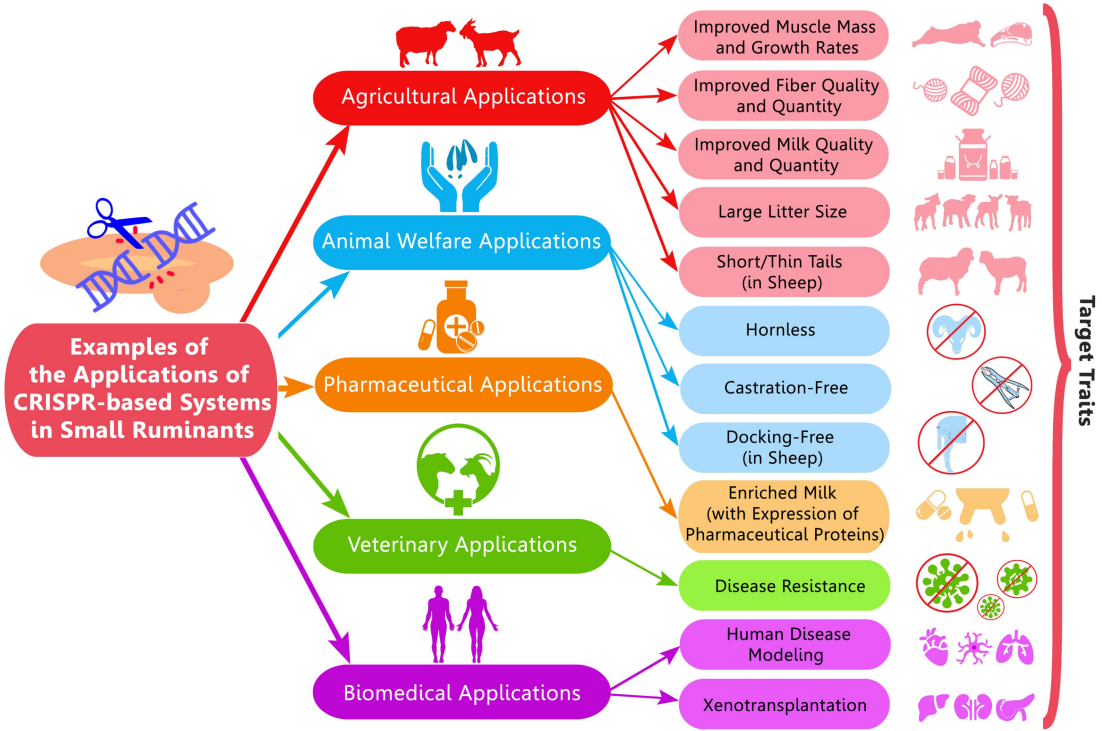


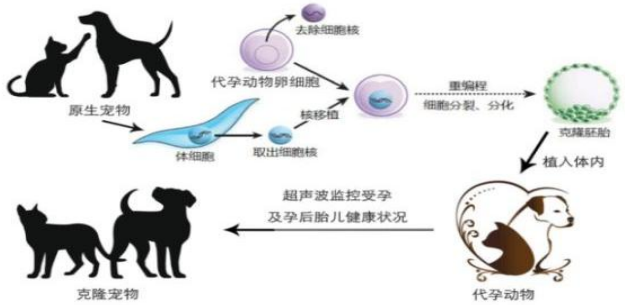
FirstGenetically Modified Pig Liver Transplant to human



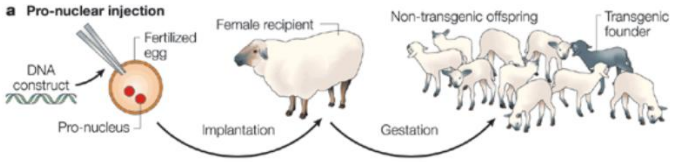
Pigs for xenotransplantation

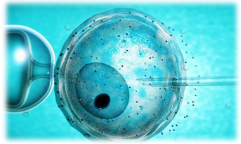
Animal Engineering and Breeding





By Somatic cell editing and cloning





By embryo microinjection

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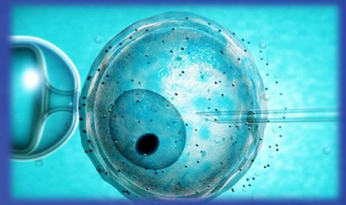
基因编辑婴儿:
Gene-edited babies-
伦理之殇
Tragedy of Ethics in Research

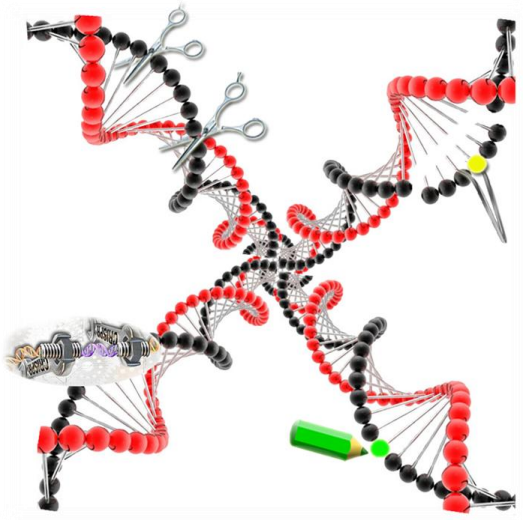


Once caused huge controversy in public

Assignments and Discussions:

1. 请列举5种以上基于不同基因编辑工具的基因敲除策略，并简述其基本原理; Please list more than five gene knockout strategies based on different gene editing tools and briefly introduce their basic molecular principles

2. 试论述制备基因编辑动物的是和制备基因编辑婴儿的非; Try to discuss the different ethical considerations in the development of Genetically Edited Animals vs. Genetically Edited human Infants



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NORTHWEST A&F UNIVERSITY