



生物育种专业·基因编辑技术课程

第四章：马良神笔—碱基编辑器Base editors

徐坤 副教授

QQ: 564737724 Tel:17792639752 2024.10.31



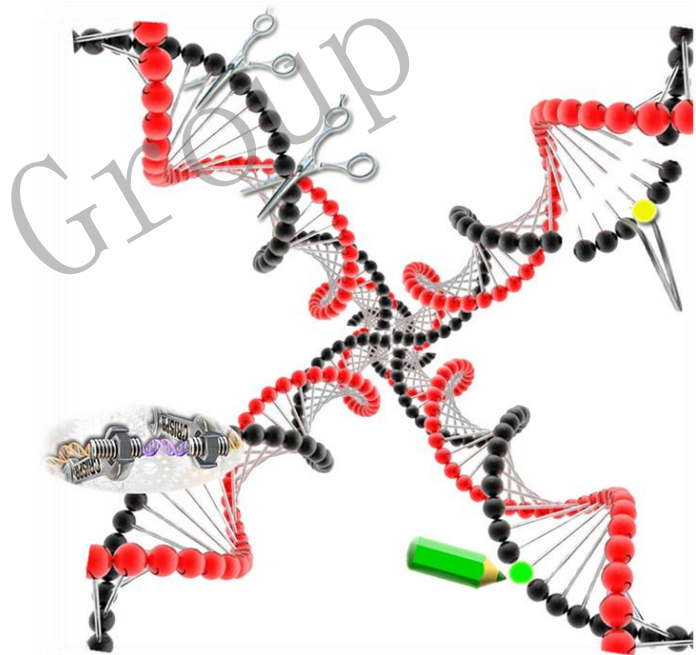
西北农林科技大学



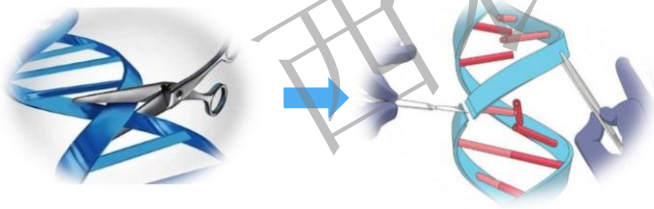
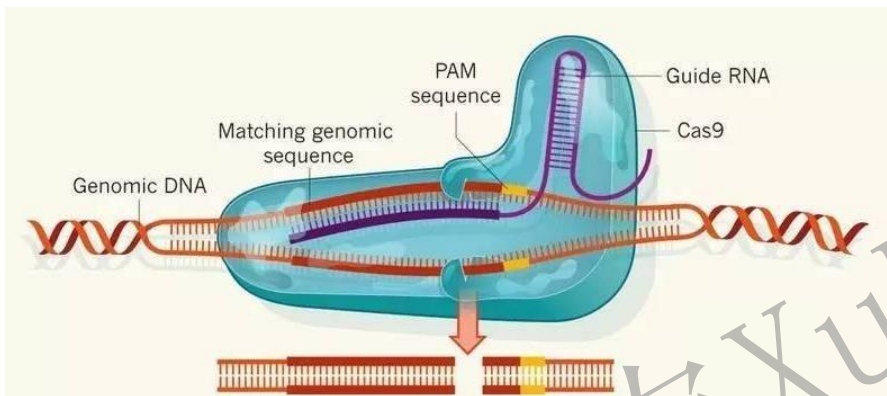
上节课拓展思考：

除了NHEJ, MMEJ, HR和SSA, 还有
哪些DNA损伤的修复机制, 可否用于
基因编辑?

不切断DNA, 似乎遗传安全性更高
还有别的策略进行基因编辑吗?



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



马良的神笔

碱基编辑器 Base Editors



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05 老木逢春



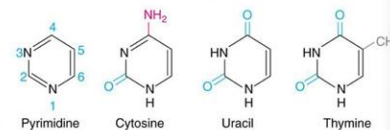
“偷天换日”就是“偷换天日”的意思。天上的太阳，众目所瞩，竟然可以把太阳给偷换掉，比喻改变重大事物真相，**技巧精妙**。

(1) 碱基 (Bases)

Bicyclic
嘌呤碱
Purines:



Monocyclic
嘧啶碱
pyrimidine:



Thymine (T) is a 5-methyluracil (U) 2-11

偷天换日之CBEs (Cytosine Base Editors)

> 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/>

2017年, **David Liu**入选 “Nature年度十大人物”

单碱基编辑技术被评为 “Science年度十大突破”

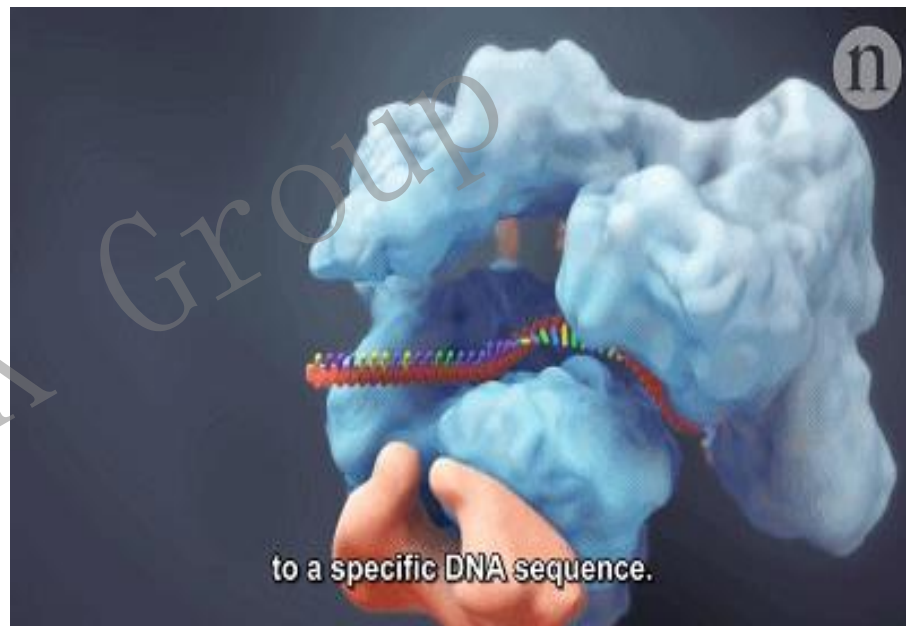
BE1: rAPOBEC1-dCas9

BE2: rAPOBEC1-dCas9-UGI

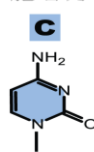
BE3: rAPOBEC1-nCas9-UGI

BE4: rAPOBEC1-nCas9-2×UGI

.....



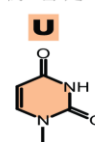
胞嘧啶



胞嘧啶
脱氨酶

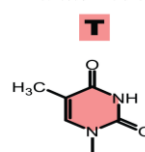


尿嘧啶



DNA聚合酶

胸腺嘧啶



西北农林科技大学

偷天换日之CBEs (Cytosine Base Editors)

> *Science*. 2016 Sep 16;353(6305):aaf8729. doi: 10.1126/science.aaf8729. Epub 2016 Aug 4.

Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems

Keiji Nishida¹, Takayuki Arazoe¹, Nozomu Yachie², Satomi Banno¹, Mika Kakimoto¹, Mayura Tabata¹, Masao Mochizuki¹, Aya Miyabe¹, Michihiro Araki¹, Kiyotaka Y Hara³, Zenpei Shimatani¹, Akihiko Kondo⁴

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Target-AID

Affiliations

¹ Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan.

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

日本神户大学
Akihiko Kondo(近藤昭彦)

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



西北农林科技大学

> *Nat Methods*. 2016 Dec;13(12):1029-1035. doi: 10.1038/nmeth.4027. Epub 2016 Oct 10.

Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells

Yunqing Ma¹, Jiayuan Zhang¹, Weijie Yin¹, Zhenchao Zhang¹, Yan Song², Xing Chang^{1,3}

Affiliations — collapse

Affiliations

dCas9-AID

¹ Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences & Shanghai Jiao Tong University School of Medicine (SJTUSM), Chinese Academy of Sciences, Shanghai, China.

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

中科院, 常兴
2018年加入西湖大学



偷天换日之ABEs (Adenine Base Editors)

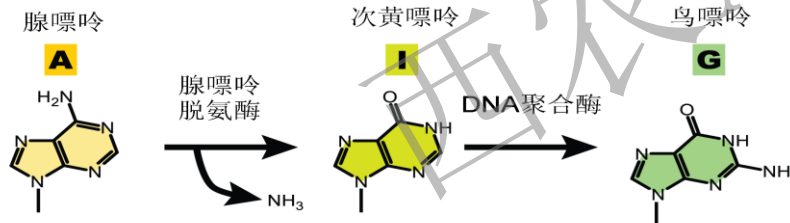
Comparative Study > Nature. 2017 Nov 23;551(7681):464-471. doi: 10.1038/nature24644.

Epub 2017 Oct 25.

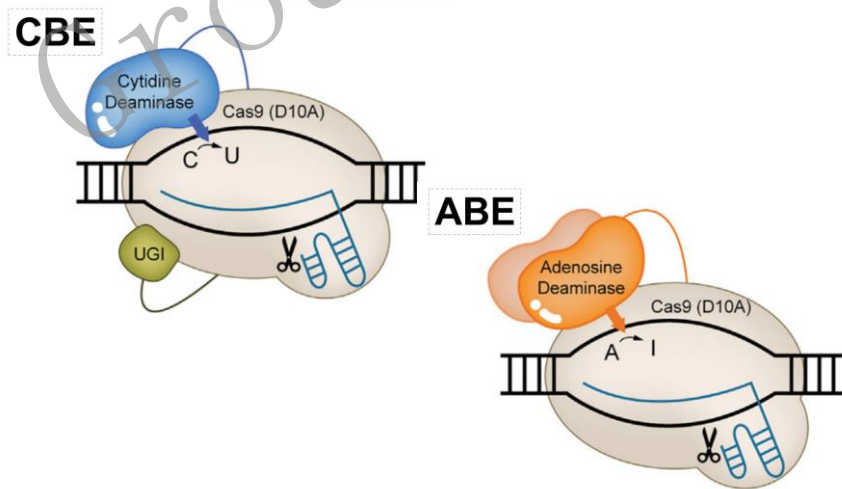
Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

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: 大肠杆菌的tRNA腺苷脱氨酶



ABE7.10(ecTadA-ecTadA*-nCas9)



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偷天换日之 ABEs (Adenine Base Editors)

□ Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity.

1 Richter MF, Zhao KT, Eton E, Lapinaite A, Newby GA, Thuronyi BW, Wilson C, Koblan LW, Zeng J, Bauer DE, Doudna JA, Liu DR.

Nat Biotechnol. 2020 Jul;38(7):883-891. doi: 10.1038/s41587-020-0453-z. Epub 2020 Mar 16.

PMID: 32433547 [Free PMC article.](#)

ABE8e offers substantially improved editing efficiencies when paired with a variety of Cas9 or Cas12 homologs. ...ABE8e augments the effectiveness and applicability of adenine base editing....

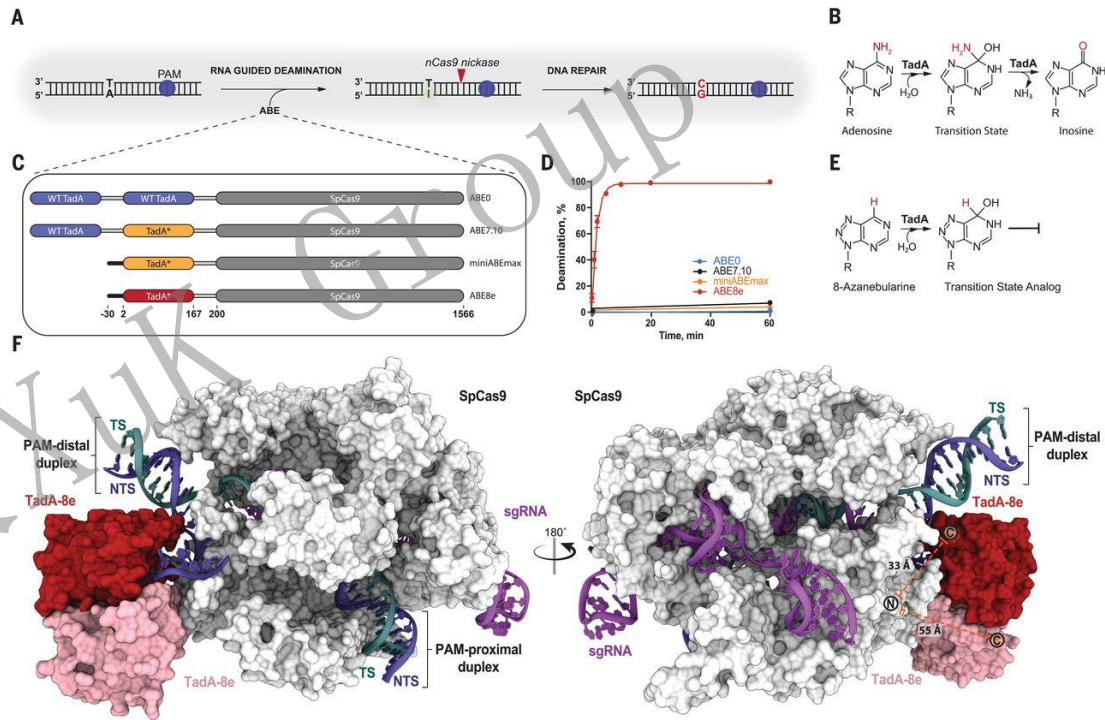
□ DNA capture by a CRISPR-Cas9-guided adenine base editor.

2 Lapinaite A, Knott GJ, Palumbo CM, Lin-Shiao E, Richter MF, Zhao KT, Beal PA, Liu DR, Doudna JA.

Science. 2020 Jul 31;369(6503):566-571. doi: 10.1126/science.abb1390.

PMID: 32732424 [Free PMC article.](#)

Furthermore, ABE8e's accelerated DNA deamination suggests a previously unobserved transient DNA melting that may occur during double-stranded DNA surveillance by CRISPR-Cas9. These results explain ABE8e-mediated base-editing outcomes and inform the future design of ...

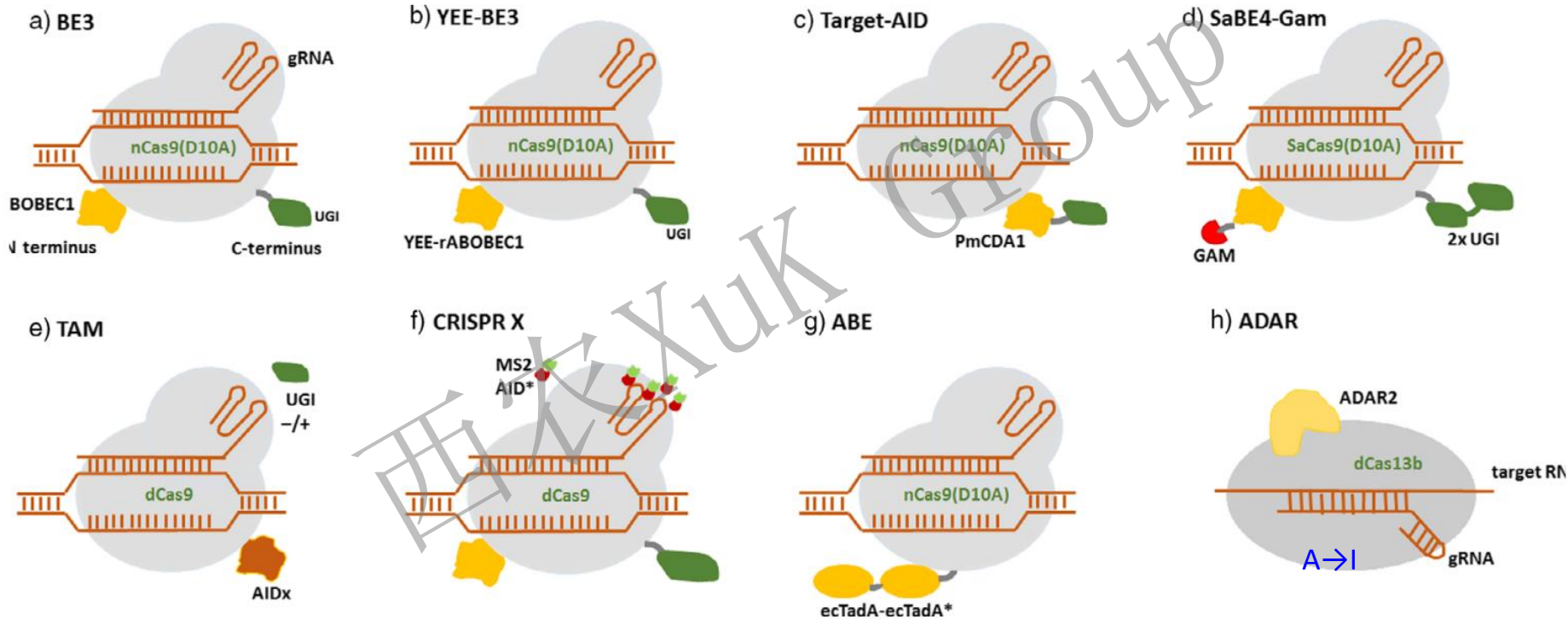


ABE8e: TadA8e-nCas9
ABE8e-V106W



西北农林科技大学

偷天换日之dBEs (deaminase-based Base Editors)



偷天换日之dBEs (deaminase-based Base Editors)

<https://pubmed.ncbi.nlm.nih.gov/29553573/>

> Nat Biotechnol. 2018 Apr;36(4):324-327. doi: 10.1038/nbt.4102. Epub 2018 Mar 19.

Base editing with a Cpf1-cytidine deaminase fusion

Xiaosa Li^{1 2 3}, Ying Wang⁴, Yajing Liu^{1 2 3}, Bei Yang⁵, Xiao Wang^{1 2 3}, Jia Chen¹, Zongyang Lu^{1 2 3}, Yuxi Zhang¹, Jing Wu¹, Xingxu Huang¹, Li Yang⁴, Ji Chen¹

Affiliation

PMID: 29553573 DOI: 10.1038/nbt.4102

上海科技大学 陈佳

南大/上科大/浙大/黄行许

> Nat Biotechnol. 2018 Nov;36(10):946-949. doi: 10.1038/nbt.4198. Epub 2018 Aug 20.

Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion

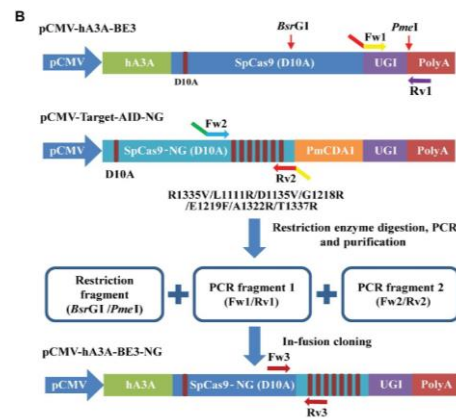
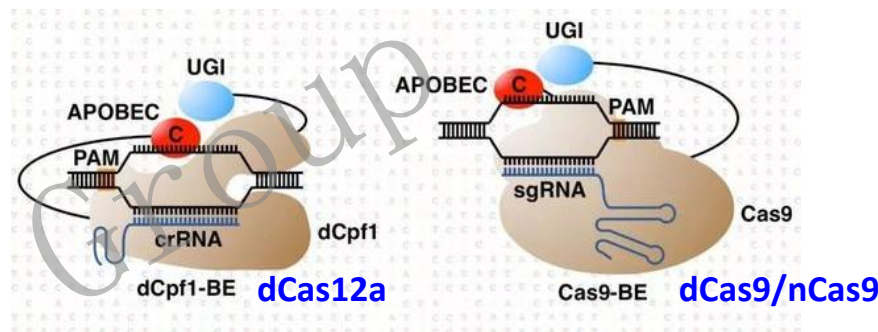
Xiao Wang^{1 2 3}, Jianan Li^{1 2 3}, Ying Wang⁴, Bei Yang⁵, Jia Wei⁴, Jing Wu¹, Ruixuan Wang¹, Xingxu Huang¹, Jia Chen¹, Li Yang⁴

Affiliations + expand

PMID: 30125268 DOI: 10.1038/nbt.4198

中科院/现复旦大学

<https://pubmed.ncbi.nlm.nih.gov/30125268/>



偷天换日之dBEs (deaminase-based Base Editors)

遗传 Hereditas (Beijing) 2019年9月, 41(9): 777-800
www.chinagene.cn

综述

碱基编辑系统研究进展

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中国科学院遗传与发育生物学研究所, 植物细胞与染色体工程国家重点实验室, 基因组编辑中心, 北京 100101



高彩霞--中科院遗传发育所

表 2 各种碱基编辑器(BE)的特点

Table 2 Base editors with various characteristics

BE 名称	脱氨酶	Cas 蛋白	PAM 类型	UGI 个数	NLS 个数	密码子 优化	编辑 活性窗口	参考 文献
BE1	rAPOBE1	dCas9	NGG	-	1x	-	4-8	[18]
BE2	rAPOBE1	dCas9	NGG	1x	1x	-	4-8	[18]
BE3	rAPOBE1	nCas9	NGG	1x	1x	-	4-8	[18]
PBE	rAPOBE1	nCas9	NGG	1x	3x	+	3-9	[82]
HF-BE3	rAPOBE1	HF1-nCas9	NGG	1x	1x	-	4-8	[123]
HF2-BE2	rAPOBE1							
BE4	rAPOBE1							
BE4-Gam	rAPOBE1							
BE4max	rAPOBE1							
AncBE4max	rAPOBE1							
eBE-S3	rAPOBE1							
FNLS-BE3	rAPOBE1							
VQR-BE3	rAPOBE1							
EQR-BE3	rAPOBE1							
VRER-BE3	rAPOBE1							
SABE3	rAPOBE1							
SABE4	rAPOBE1							
SABE4-Gam	rAPOBE1							
SaKKH-BE3	rAPOBE1							
Spy-mnCas9-BE3	rAPOBE1							
dCpf1-eBE	rAPOBE1							
gAaBE1-L1.4	rAPOBE1							
xBE3	rAPOBE1							
BE3-NG	rAPOBE1							
YE1-BE3	rAPOBE1-YE1	nCas9	NGG	1x	1x	-	4-7	[29]
YE2-BE3	rAPOBE1-YE2	nCas9	NGG	1x	1x	-	5-6	[29]
EE-BE3	rAPOBE1-EE	nCas9	NGG	1x	1x	-	5-6	[29]
YEE-BE3	rAPOBE1-YEE	nCas9	NGG	1x	1x	-	5-6	[29]
BE3-PAPAPAP	rAPOBE1	nCas9	NGG	1x	1x	-	5-6	[36]
BE3-R33A	rAPOBE1-R33A	nCas9	NGG	1x	1x	-	5-7	[131]
BE3-R33A/K34A	rAPOBE1-R33A/K34A	nCas9	NGG	1x	1x	-	5-6	[131]
BE-PLUS	rAPOBE1-seFV	nCas9	NGG	1x	1x	-	4-16	[37]
CP-CBEmax	rAPOBE1	CP-nCas9	NGG	2x	1x	-	4-11	[25]
eA3A-BE3	hAPOBE3A-N57G	nCas9	NGG	1x	1x	-	4-8	[41]
eA3A-HF1-BE3-2xUGI	hAPOBE3A-N57G	HF1-nCas9	NGG	2x	1x	-	4-8	[41]
eA3A-Hypa-BE3-2xUGI	hAPOBE3A-N57G	nHypaCas9	NGG	2x	1x	-	4-8	[41]
hA3A-BE3	hAPOBE3A	nCas9	NGG	1x	1x	-	2-13	[38]
hA3A-BE3-Y130F	hAPOBE3A-Y130F	nCas9	NGG	1x	1x	-	3-8	[38]
A3A-PBE	hAPOBE3A	nCas9	NGG	1x	2x	+	1-17	[28]

续表

BE 名称	脱氨酶	Cas 蛋白	PAM 类型	UGI 个数	NLS 个数	密码子 优化	编辑 活性窗口	参考 文献
DBE-A3A	hAPOBE3A-MS2	nCas9	NGG	1x	1x	-	2-17	[39]
hA3A ^{122A}	hAPOBE3A-R122A	nCas9	NGG	1x	1x	-	3-9	[133]
A3G-BE4max	hAPOBE3G-CTD	nCas9	NGG	2x	1x	-	4-10	[42]
Target-AID	PmCDA1	nCas9	NGG	1x	1x	-	2-8	[19]
Target-AID-NG	PmCDA1	nCas9	NG	1x	1x	-	2-8	[35]
								[36]
								[20]
								[21]
								[40]
								[39]
								[22]
								[22]
								[107]
								[107]
								[108]
								[26]
								[30,114]
								[25]
								[30]
								[25]
								[111]
								[25]
								[30,117]
SaKKH-ABE	TadA-TadA*	nSaKKH-Cas9	NNRRRT	-	1x	+	8-13	[25]
ABE3	TadA-TadA*	nCas9	NG	-	1x	+	4-14	[25]
xABE	TadA-TadA*	nCas9	NG	-	1x	+	4-7	[34]
xABEEmax	TadA-TadA*	mxCas9	NG	-	2x	+	4-8	[25]
ABE-NG	TadA-TadA*	nSpCas9-NG	NG	-	1x	+	4-7	[74]
ABE-NG-S	TadA*	nSpCas9-NG	NG	-	1x	+	4-7	[74]
NG-ABEEmax	TadA-TadA*	nSpCas9-NG	NG	-	2x	+	4-8	[35]
CP-ABEEmax	TadA-TadA*	CP-nCas9	NGG	-	2x	+	4-12	[25]
ScCas9-ABE(7.10)	TadA-TadA*	nScCas9	NNGN	-	1x	+	4-7	[109]
ABEEmaxAW	TadA-E59A-TadA*-V106W	nCas9	NGG	-	2x	+	4-8	[132]
ABEEmaxQW	TadA-E59Q-TadA*-V106W	nCas9	NGG	-	2x	+	4-8	[132]
ABE7.10 ^{118A}	TadA-F148A-TadA*-F148A	nCas9	NGG	-	1x	-	5	[133]

> Cell. 2023 Jul 20;186(15):3182-3195.e14. doi: 10.1016/j.cell.2023.05.041. Epub 2023 Jun 27.

Discovery of deaminase functions by structure-based protein clustering

Jiaying Huang¹, Qiupeng Lin¹, Hongyuan Fei², Zixin He², Hu Xu³, Yunjia Li², Kunli Qu⁴, Peng Han⁴, Qiang Gao³, Boshu Li², Guanwen Liu¹, Lixiao Zhang³, Jiacheng Hu¹, Rui Zhang¹, Erwei Zuo⁵, Yonglun Luo⁶, Yidong Ran³, Jin-Long Qiu⁷, Kevin Tianmeng Zhao⁸, Caixia Gao⁹

Affiliations — collapse

<https://pubmed.ncbi.nlm.nih.gov/37379837/>

DddA-like DNA deaminases

偷天换日之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 (...)

☆ Save ↀ Cite Cited by 597 Related articles All 7 versions

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** ...

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HTM1J 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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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 ...)

☆ Save ↀ Cite Cited by 2 Related articles All 7 versions

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 ...)

☆ 保存 ↀ 引用 被引用次数: 32 相关文章 所有 3 个版本

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 ↀ 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 ...

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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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目录

01 偷天换日

02 托梁换柱

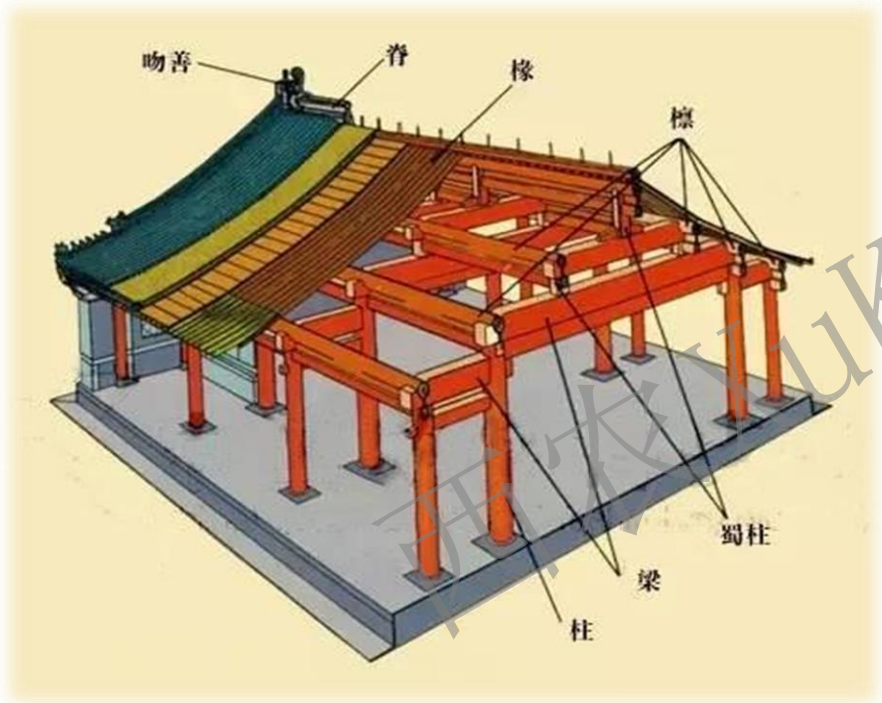
03 抽梁换柱

04 双剑合璧

05 老木逢春



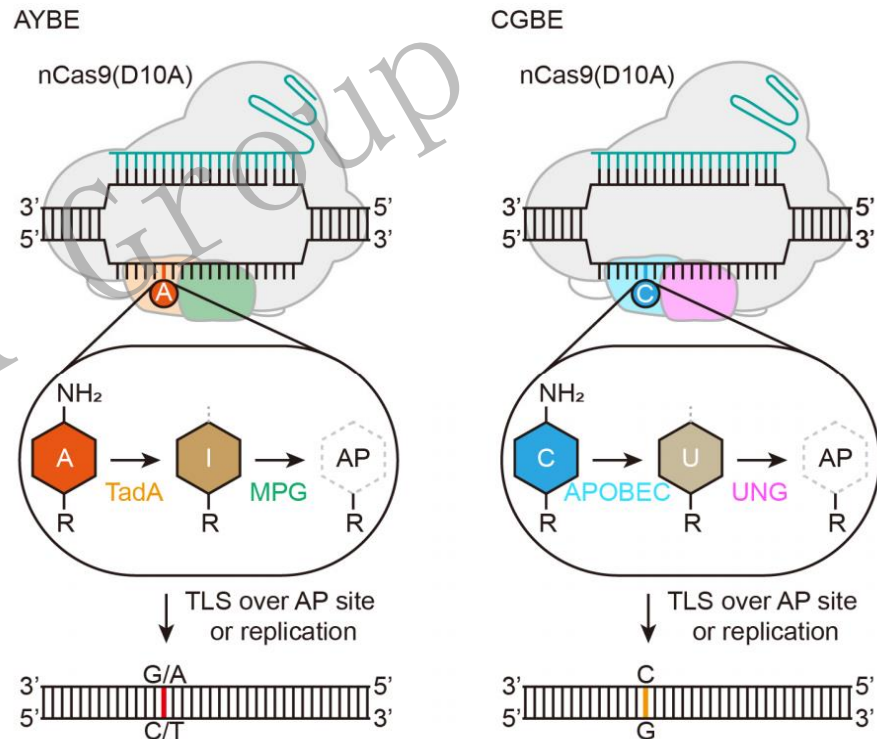
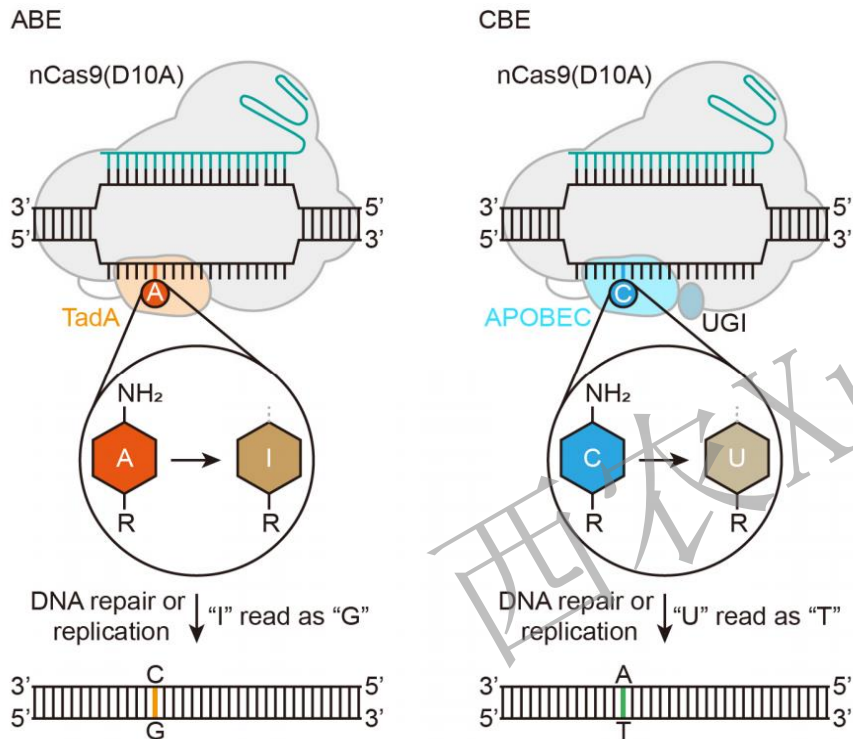
托梁换柱



在古建筑领域中，“托梁换柱”作为用于残损木柱加固的科学方法，不仅加固效果好，而且对建筑稳定性影响小，是我国古代工匠建筑智慧的反映。



托梁换柱之 dgBEs (deaminase & glycosylase-based Base Editors)



dBES: deaminase-based Base Editors



西北农林科技大学

dgBEs

托梁换柱之dgBEs--CGBE

<https://pubmed.ncbi.nlm.nih.gov/32690970/>

> Nat Biotechnol. 2021 Jan;39(1):35-40. doi: 10.1038/s41587-020-0592-2. Epub 2020 Jul 20.

Glycosylase base editors enable C-to-A and C-to-G base changes

Dongdong Zhao ^{#1}, Ju Li ^{#2}, Siwei Li ^{#1}, Xiuqing Xin ^{1 3}, Muzi Hu ^{1 4}, Marcus A Price ⁵, Susan J Rosser ⁵, Changhao Bi ⁶, Xueli Zhang ⁷

Affiliations – collapse

Affiliations

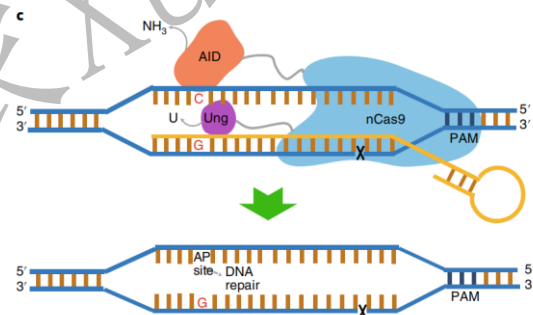
¹ Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China.

Glycosylase base editors (GBEs)

- **CABE in *E. coli***
(AID-nCas9-Ung)
- **CGBE in mammalian cells**
(APOBEC-nCas9-Ung)



中科院天工所张学礼



西北农林科技大学



生命科学领域颠覆性新技术-新型糖基化酶
碱基编辑器 (GBE)

2020年10月21日 上午10:00



毕昌昊 研究员

中国科学院天津工业生物技术研究所
合成生物技术项目组PI
研究方向: 合成生物学技术与基因编辑技术

课程亮点

1. CRISPR碱基编辑基本原理
2. 新型碱基编辑器开发
3. 碱基编辑器在细胞生物学研究和遗传疾病治疗中的应用

扫码报名



直播福利

1. 预报名前10名可免费获得1个该编辑器质粒 pAPOBEC_nCas9_Ung (仅限学术客户)
2. 所有顾客均可参与MolecularCloud 限时免费单, 砍价0元购的活动获得免费质粒

MolecularCloud

中科院天工所毕昌昊

托梁换柱之 dgBEs--CGBE

> Nat Biotechnol. 2021 Jan;39(1):41-46. doi: 10.1038/s41587-020-0609-x. Epub 2020 Jul 20.

CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells

Ibrahim C Kurt # 1 2 3, Ronghao Zhou # 1 2, Sowmya Iyer 1, Sara P Garcia 1, Bret R Miller 1 2, Lukas M Langner 1 2, Julian Grünewald 4 5 6, J Keith Joung 7 8 9

Affiliations — collapse

Affiliations

1 Molecular Pathology Unit, Massachusetts General Hospital, Charlestown, MA, USA.

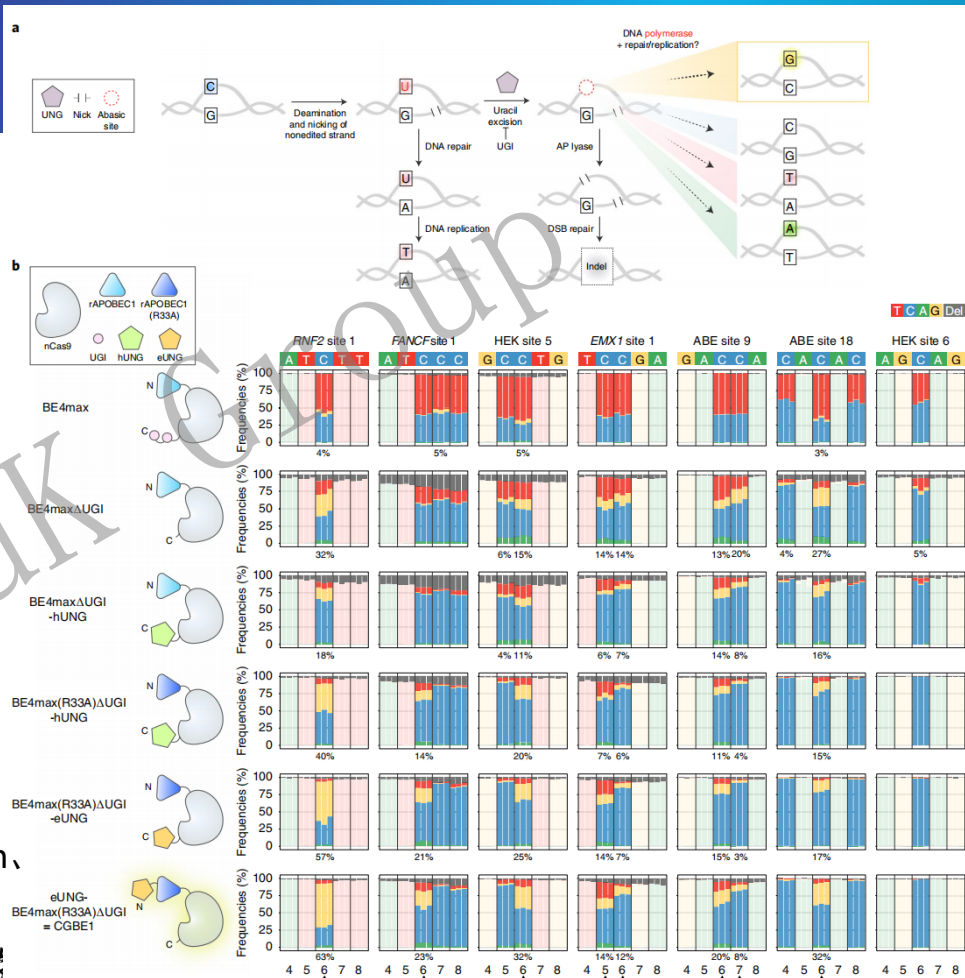
<https://pubmed.ncbi.nlm.nih.gov/32690971/>

华人学者?
J. Keith Joung

基因编辑公司 Editas :
JK Joung、张锋、George Church、
Jennifer Doudna、刘如谦



西北大学



托梁换柱之 dgBEs--CGBE

> Nat Biotechnol. 2021 Nov;39(11):1414-1425. doi: 10.1038/s41587-021-00938-z. Epub 2021 Jun 28.

Efficient C•G-to-G•C base editors developed using CRISPRi screens, target-library analysis, and machine learning

Luke W Koblan # 1 2 3, Mandana Arbab # 1 2 3, Max W Shen # 1 2 3 4, Jeffrey A Hussmann # 5 6 7 8 9, Andrew V Anzalone # 1 2 3, Jordan L Doman # 1 2 3, Gregory A Newby # 1 2 3, Dian Yang # 5 7 8 9, Beverly Mok # 1 2 3, Joseph M Replogle # 5 7 10 11 8 9, Albert Xu # 5 6 10 12, Tyler A Sisley # 2, Jonathan S Weissman # 13 14 15 16 17, Britt Adamson # 18 19 20 21, David R Liu # 22 23 24

<https://pubmed.ncbi.nlm.nih.gov/34183861/>

> Nat Commun. 2021 Aug 12;12(1):4902. doi: 10.1038/s41467-021-25217-y.

Optimization of C-to-G base editors with sequence context preference predictable by machine learning methods

Tanglong Yuan # 1, Nana Yan # 1, Tianyi Fei # 2, Jitan Zheng # 1 3, Juan Meng # 2, Nana Li # 1, Jing Liu # 1, Haihang Zhang # 1, Long Xie # 1, Wenqin Ying # 2, Di Li # 1 4, Lei Shi # 1, Yongsun Sun # 1, Yongyao Li # 1, Yixue Li # 5, Yidi Sun # 6, Erwei Zuo # 7

Affiliations — collapse <https://pubmed.ncbi.nlm.nih.gov/34385461/>

Affiliations

- 1 Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China.

> Science. 2019 Apr 19;364(6437):289-292. doi: 10.1126/science.aav9973. Epub 2019 Feb 28.

Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos

Erwei Zuo # 1 2, Yidi Sun # 3, Wu Wei # 4 5 6, Tanglong Yuan # 2, Wenqin Ying # 1, Hao Sun # 7, Liyun Yuan # 4, Lars M Steinmetz # 8 9 10, Yixue Li # 11 12 13, Hui Yang # 14

Affiliations — collapse

<https://pubmed.ncbi.nlm.nih.gov/30819928/>

Affiliations

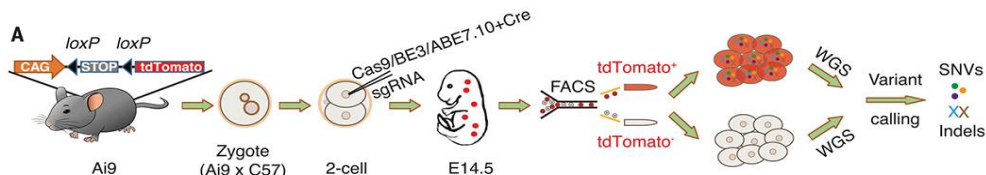
- 1 Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Research Center for Brain Science and Brain-Inspired Intelligence, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China



中科院神经所/药物所杨辉

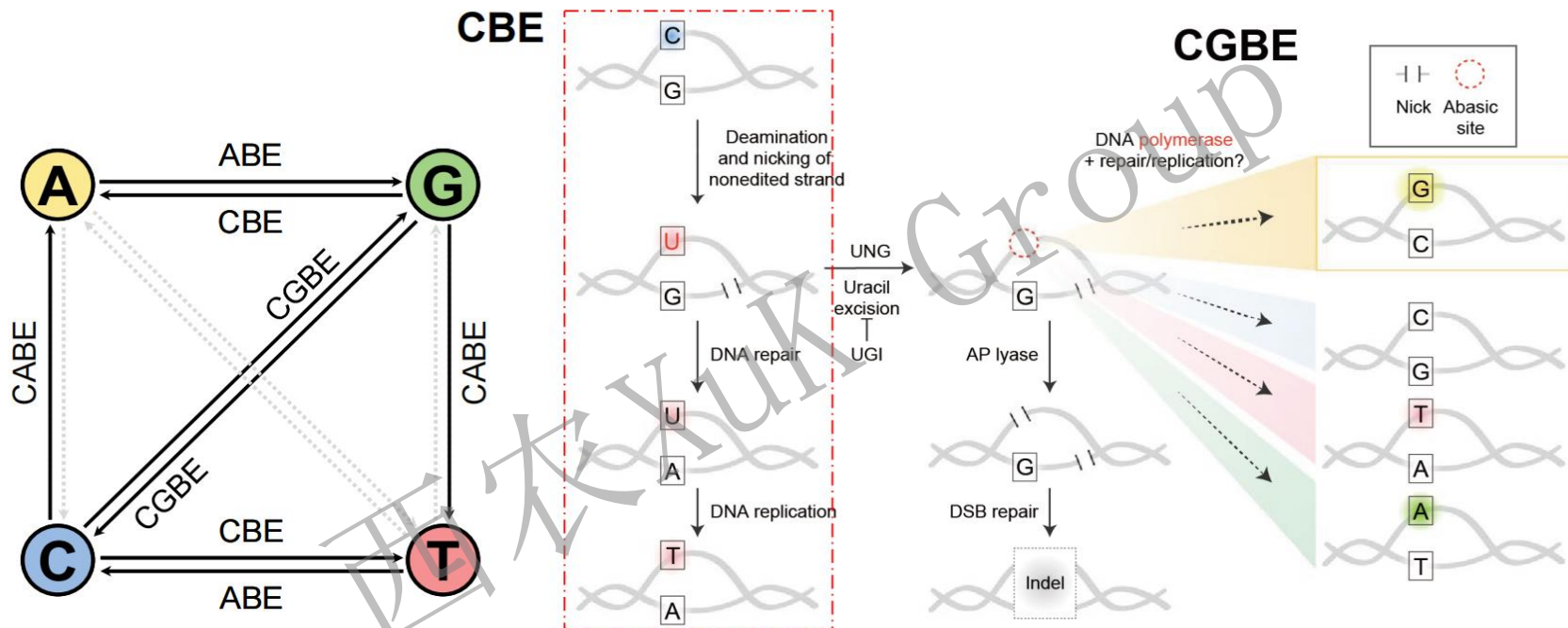


农科院深圳基因所左二伟



GOTI: 高性能基因编辑脱靶效应检测工具
(genome-wide off-target analysis by two-cell embryo injection)

托梁换柱之 dgBEs--CGBE



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



托梁换柱之 dgBEs--CGBE

> Nat Biotechnol. 2023 May;41(5):663-672. doi: 10.1038/s41587-022-01532-7. Epub 2022 Nov 10.

Re-engineering the adenine deaminase TdaA-8e for efficient and specific CRISPR-based cytosine base editing

Liang Chen ^{#1}, Biyun Zhu ^{#1}, Gaomeng Ru ^{#1}, Haowei Meng ^{#2}, Yongchang Yan ^{#2}, Mengjia Hong ¹, Dan Zhang ¹, Changming Luan ¹, Shun Zhang ¹, Hao Wu ², Hongyi Gao ¹, Sijia Bai ¹, Changqing Li ¹, Ruoyi Ding ¹, Niannian Xue ¹, Zhixin Lei ², Yuting Chen ³, Yuting Guan ¹, Stefan Siwko ⁴, Yiyun Cheng ¹, Gaojie Song ¹, Liren Wang ¹, Chengqi Yi ⁵, Mingyao Liu ^{6,7}, Dali Li ⁸

Affiliations:

<https://pubmed.ncbi.nlm.nih.gov/36357717/>

Affiliations

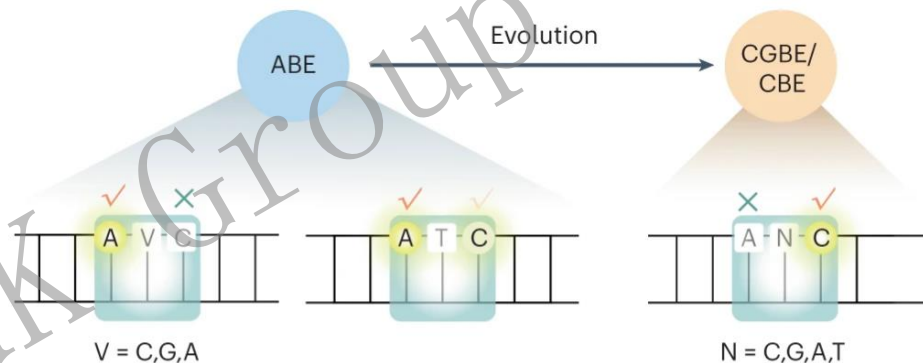
- 1 Shanghai Frontiers Science Center of Genome Editing and Cell Therapy, Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, China.



华东师范大学李大力



西北农林科技大学

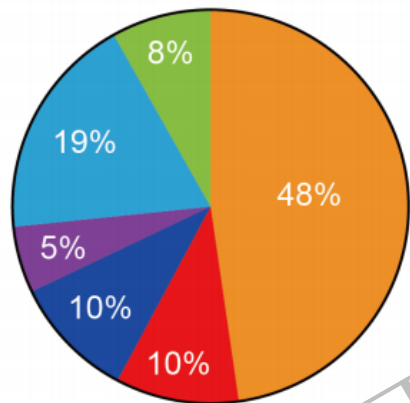


Td-CBE, Td-CGBE

DNA 单碱基编辑：已有碱基编辑工具的局限性

人类疾病相关可纠正的单碱基变异分布

(60372 total)



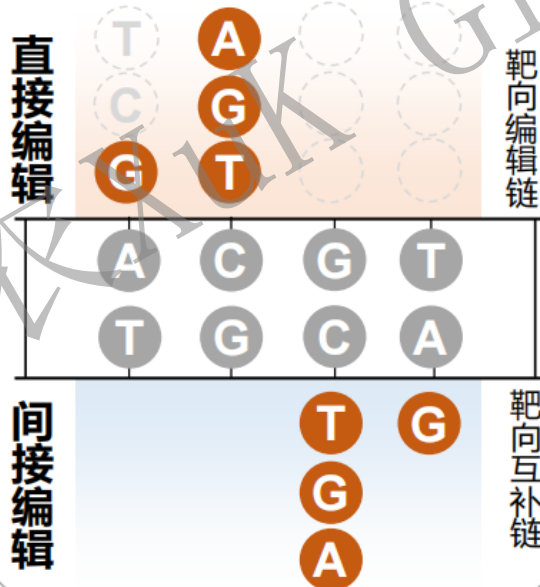
- Corrected by A.T → G.C
- Corrected by C.G → T.A
- Corrected by C.G → G.C
- Corrected by C.G → A.T
- Corrected by A.T → C.G
- Corrected by A.T → T.A

27%

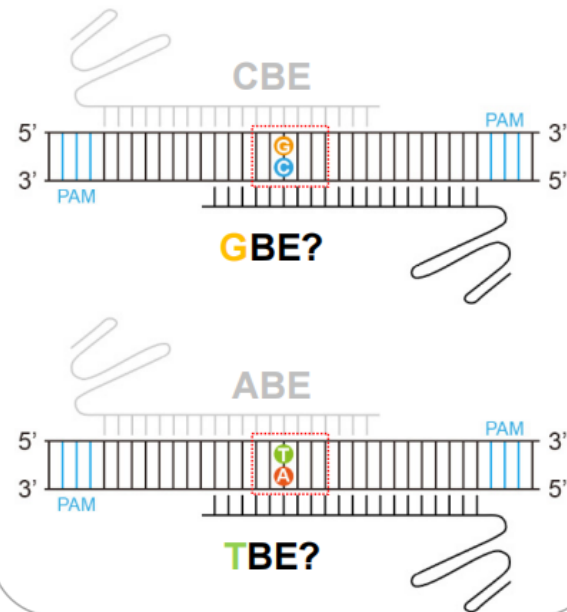
现有的 DNA 单碱基编辑工具

ABE: **A** → **G** CBE: **C** → **T** CGBE: **C** → **G A**

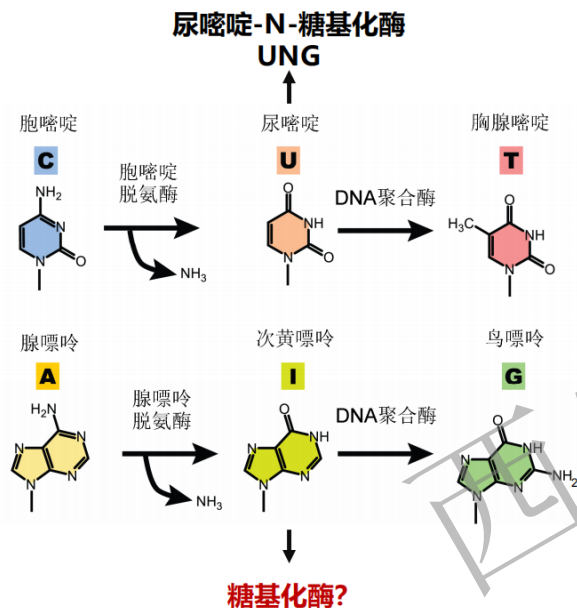
碱基编辑类型有限



PAM、编辑窗口限制



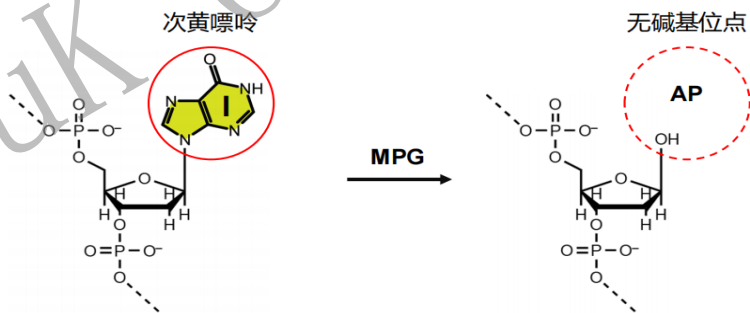
托梁换柱之 dgBEs—A?BE



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/aprimidinic site

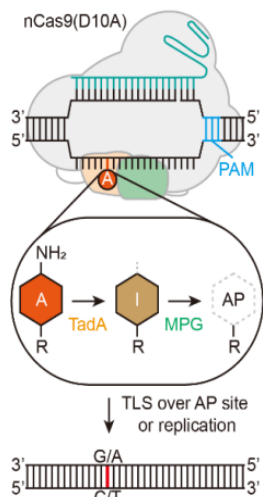


西北农林科技大学

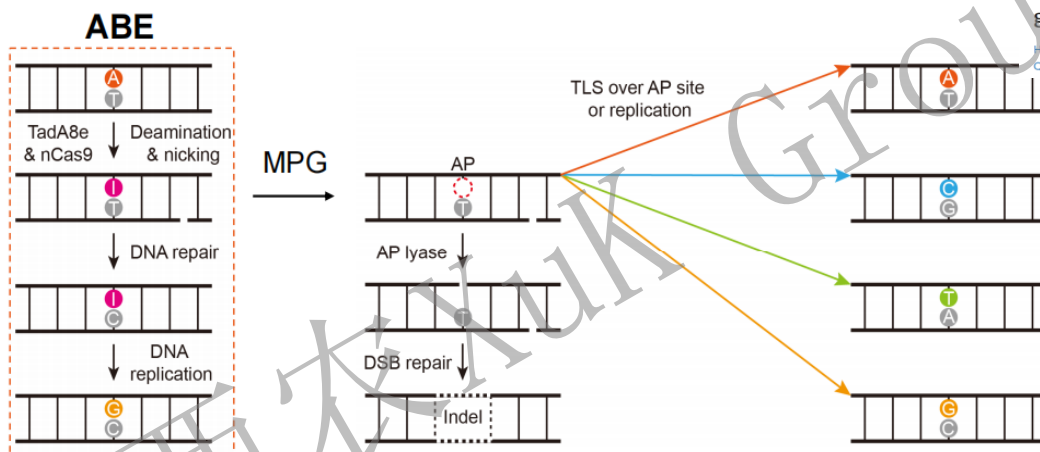
托梁换柱之 dgBEs--AYBE

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/aprimidinic site; DSB, Double-Strand Break; TLS, Translesion Synthesis.

> 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

Huiwei 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}



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/36624150/>

托梁换柱之dgBEs--AXBE/AYBE/ACBE

Adenine transversion editors enable precise, efficient A•T-to-C•G base editing in mammalian cells and embryos

Liang Chen ^{#1}, Mengjia Hong ^{#1}, Changming Luan ^{#1}, Hongyi Gao ¹, Gaomeng Ru ¹, Xinyuan Guo ¹, Dujuan Zhang ¹, Shun Zhang ¹, Changwei Li ², Jun Wu ¹, Peyton B Randolph ^{3 4 5}, Alexander A Sousa ^{3 4 5}, Chao Qu ¹, Yifan Zhu ¹, Yuting Guan ¹, Liren Wang ¹, Mingyao Liu ^{1 6}, Bo Feng ⁷, Gaojie Song ¹, David R Liu ^{3 4 5}, Dali Li ⁸

Affiliations – collapse

Affiliations

- Shanghai Frontiers Science Center of Genome Editing and Cell Therapy, Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, China.

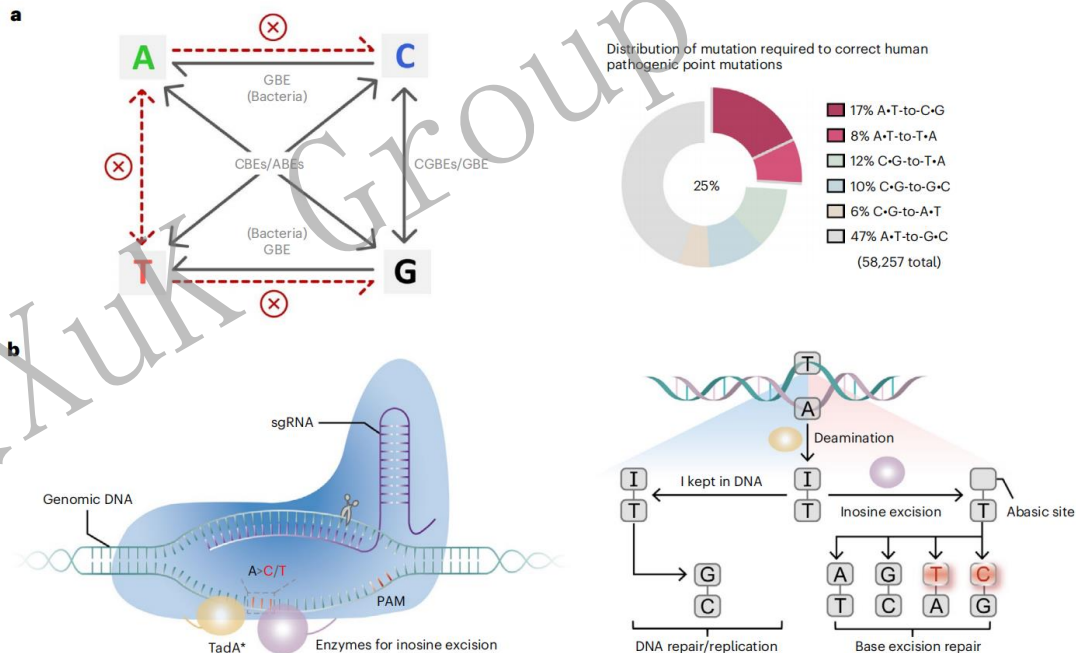
<https://pubmed.ncbi.nlm.nih.gov/37322276/>



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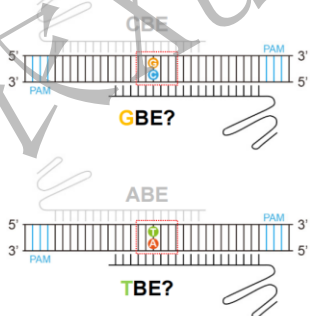
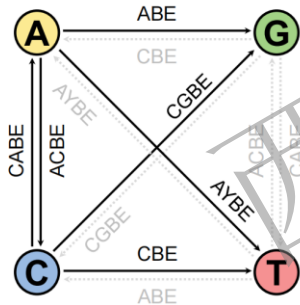
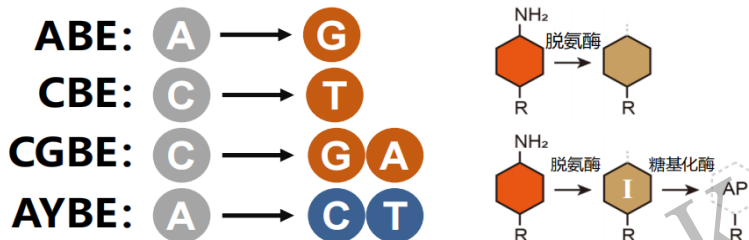


西北农林科技大学



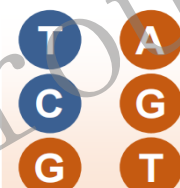
偷天换日 + 托梁换柱: dBEs + dgBEs

依赖脱氨酶的编辑工具 (A/C 脱氨反应)

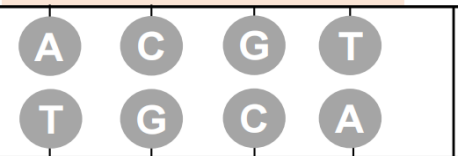


单碱基可编辑类型

直接编辑



靶向编辑链



间接编辑



靶向互补链



目录

01 偷天换日

02 托梁换柱

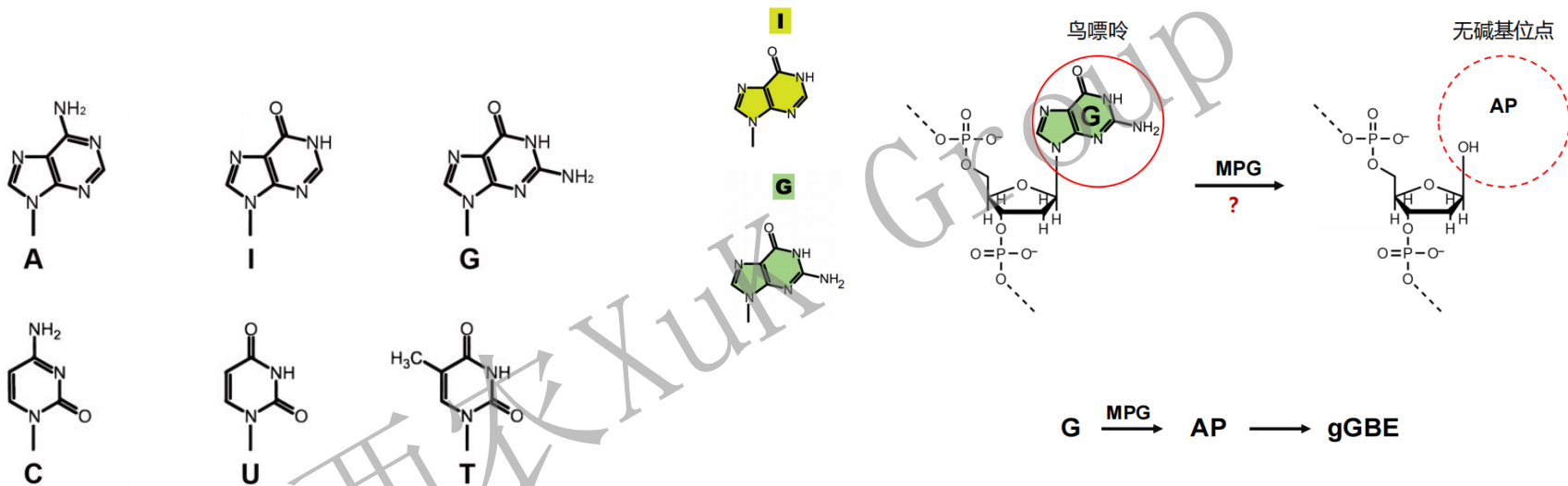
03 抽梁换柱

04 双剑合璧

05 老木逢春



抽梁换柱之gBEs (glycosylase-based Base Editors)

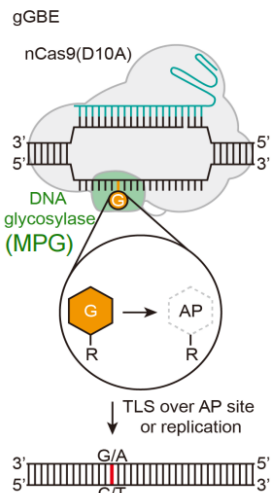


G 和 T 无法通过脱氨反应转化为其他碱基

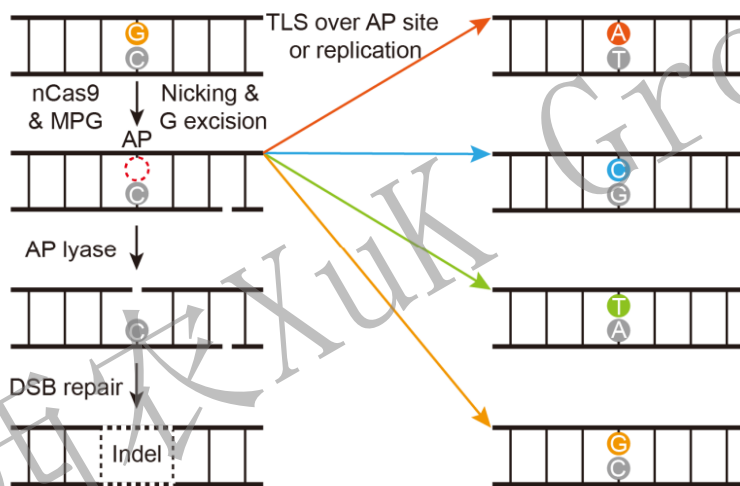


抽梁换柱之gGBE (GYBE)

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/aprimidinic site; DSB, Double-Strand Break; TLS, Translesion Synthesis.

> Natl Sci Rev. 2023 May 16;10(8):mwad143. doi: 10.1093/nsr/mwad143. eCollection 2023 Aug.

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

Huawei Tong¹, Nana Liu¹, Yinghui Wei¹, Yingsi Zhou¹, Yun Li¹, Danni Wu¹, Ming Jin², Shuna Cui¹, Hengbin Li¹, Guoling Li¹, Jingxing Zhou¹, Yuan Yuan¹, Hainan Zhang¹, Linyu Shi¹, Xian Yao¹, 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/>

抽梁换柱之gGBE (GTBE)

> J Integr Plant Biol. 2024 Jun;66(6):1048-1051. doi: 10.1111/jipb.13657. Epub 2024 Apr 5.

Targeted G-to-T base editing for generation of novel herbicide-resistance gene alleles in rice

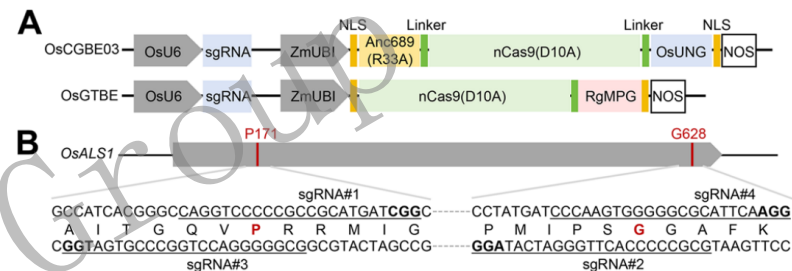
Yifu Tian^{1,2}, Xinbo Li^{1,2}, Jiyong Xie^{3,4}, Zai Zheng^{1,2}, Rundong Shen^{1,2}, Xuesong Cao⁵,
Mugui Wang¹, Chao Dong^{1,2}, Jian-Kang Zhu^{1,5}

Affiliations – collapse

Affiliations

- 1 Ministry of Agriculture and Rural Affairs Key Laboratory of Gene Editing Technologies (Hainan), Institute of Crop Sciences and National Nanfan Research Institute, Chinese Academy of Agricultural Sciences, Sanya, 572024, China.

<https://pubmed.ncbi.nlm.nih.gov/38578176/>



南方科技大学前
沿生物技术研究
院院长

中国农业科学院
基因编辑创新利
用实验室主任

朱健康院士



西北农林科技大学

抽梁换柱之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

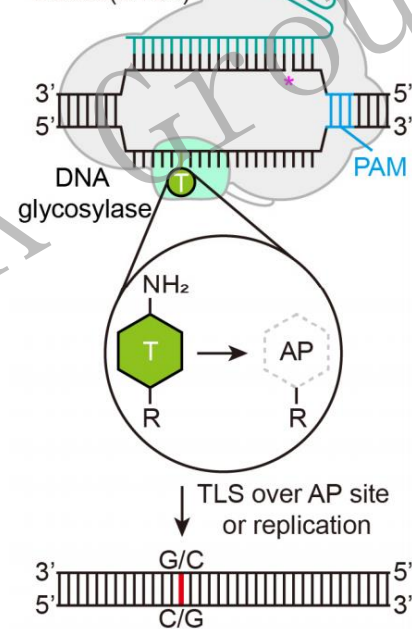
Yan He¹, Xibin Zhou², Chong Chang³, Ge Chen³, Weikuan Liu¹, Geng Li³, Xiaoqi Fan³,
Mingsun Sun³, Chensi Miao³, Qianyue Huang³, Yungqing Ma³, Fajie Yuan⁴, Xing Chang⁵

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

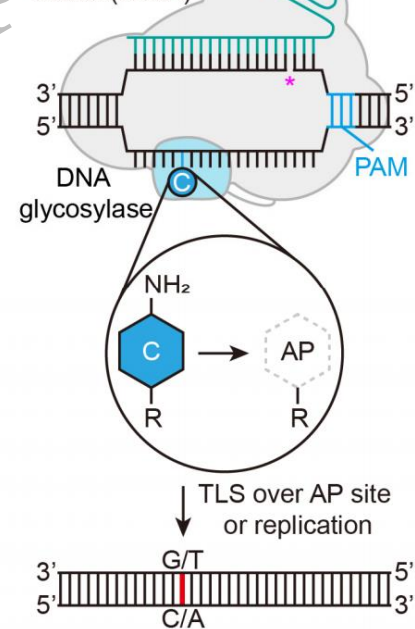
Development of deaminase-free T-to-S base editor and C-to-G base editor by engineered human uracil DNA glycosylase

Huawei Tong #¹, Haoqiang Wang #², Xuchen Wang #^{3 4}, Nana Liu #², Guoling Li²,
Danni Wu², Yun Li², Ming Jin⁵, Hengbin Li², Yinghui Wei^{6 7}, Tong Li², Yuan Yuan²,
Linyu Shi², Xuan Yao², Yingsi Zhou⁸, Hui Yang^{9 10}

gTBE
nCas9(D10A)



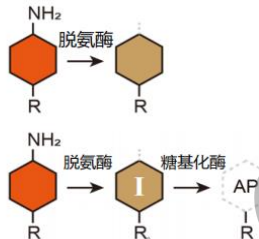
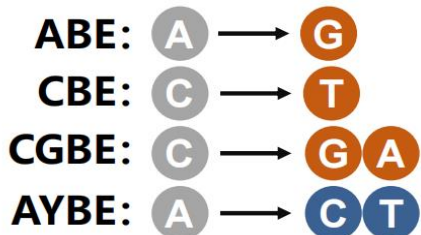
gCBE
nCas9(D10A)



基于工程化糖基化酶的碱基编辑工具大大丰富了碱基编辑类型

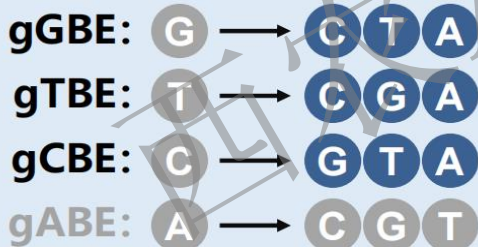
其他团队

依赖脱氨酶的编辑工具 (A/C 脱氨反应)



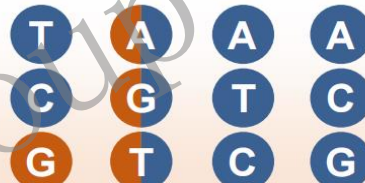
建立不依赖脱氨酶的碱基编辑策略

不依赖脱氨酶的全类型编辑工具 (碱基切除修复)

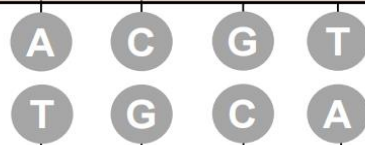


单碱基可编辑类型

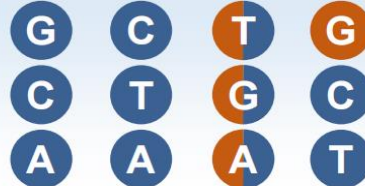
直接编辑



靶向编辑链



间接编辑



靶向互补链

童华威

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03 抽梁换柱

04 双剑合璧

05 老木逢春



双剑合璧之Dual BEs--A&CBEs



Dual base editor catalyzes both cytosine and adenine base conversions in human cells.

Zhang X, Zhu B, Chen L, Xie L, Yu W, Wang Y, Li L, Yin S, Yang L, Hu H, Han H, Li Y, Wang L, Chen G, Ma X, Geng H, Huang W, Pang X, Yang Z, Wu Y, Siwko S, Kurita R, Nakamura Y, Yang L, Liu M, Li D.
Nat Biotechnol. 2020 Jul;38(7):856-860. doi: 10.1038/s41587-020-0527-y. Epub 2020 Jun 1.

李大力: A&C-BEmax

A dual-deaminase CRISPR base editor enables concurrent adenine and cytosine editing.

Grünewald J, Zhou R, Lareau CA, Garcia SP, Iyer S, Miller BR, Langner LM, Hsu JY, Aryee MJ, Joung JK.
Nat Biotechnol. 2020 Jul;38(7):861-864. doi: 10.1038/s41587-020-0535-y. Epub 2020 Jun 1.
PMID: 32483364 [Free PMC article.](#)

JK Joung: SPACE

Base editors for simultaneous introduction of C-to-T and A-to-G mutations.

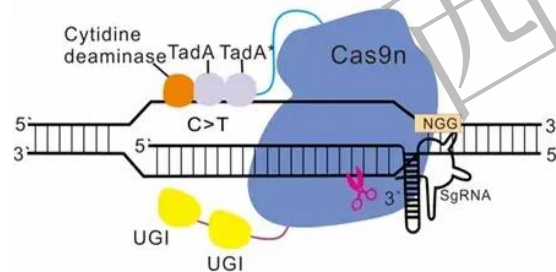
Sakata RC, Ishiguro S, Mori H, Tanaka M, Tatsuno K, Ueda H, Yamamoto S, Seki M, Masuyama N, Nishida K, Nishimasu H, Arakawa K, **Kondo A**, Nureki O, Tomita M, Aburatani H, Yachie N.
Nat Biotechnol. 2020 Jul;38(7):865-869. doi: 10.1038/s41587-020-0509-0. Epub 2020 Jun 2.
PMID: 32483365

A Kondo: Target-ACEmax

Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors.

Li C, Zhang R, Meng X, Chen S, Zong Y, Lu C, Qiu JL, Chen YH, Li J, Gao C.
Nat Biotechnol. 2020 Jul;38(7):875-882. doi: 10.1038/s41587-019-0393-7. Epub 2020 Jan 13.
PMID: 31932727

高彩霞: STEME



西北农林科技大学

双剑合璧之Dual BEs--GGBE

> Nat Commun. 2023 Apr 27;14(1):2430. doi: 10.1038/s41467-023-38193-2.

HMGN1 enhances CRISPR-directed dual-function A-to-G and C-to-G base editing

Chao Yang^{1,2}, Zhenzhen Ma³, Keshan Wang⁴, Xingxiao Dong⁵, Meiyu Huang⁶, Yaqiu Li^{1,2},
Xiagu Zhu⁷, Ju Li⁸, Zhihui Cheng³, Changhao Bi^{9,10}, Xueli Zhang^{11,12}

<https://pubmed.ncbi.nlm.nih.gov/37105976/>

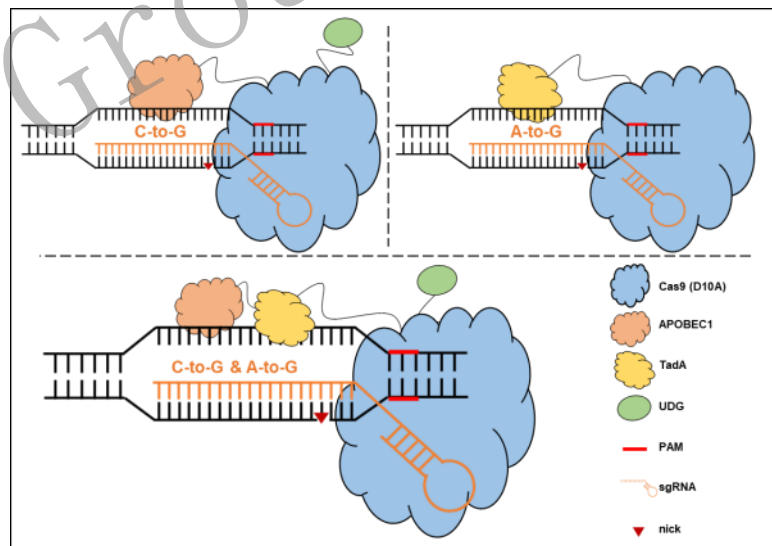


中科院天工所张学礼



中科院天工所毕昌昊

A-to-G and C-to-G base editor (GGBE)



西北农林科技大学

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03 抽梁换柱

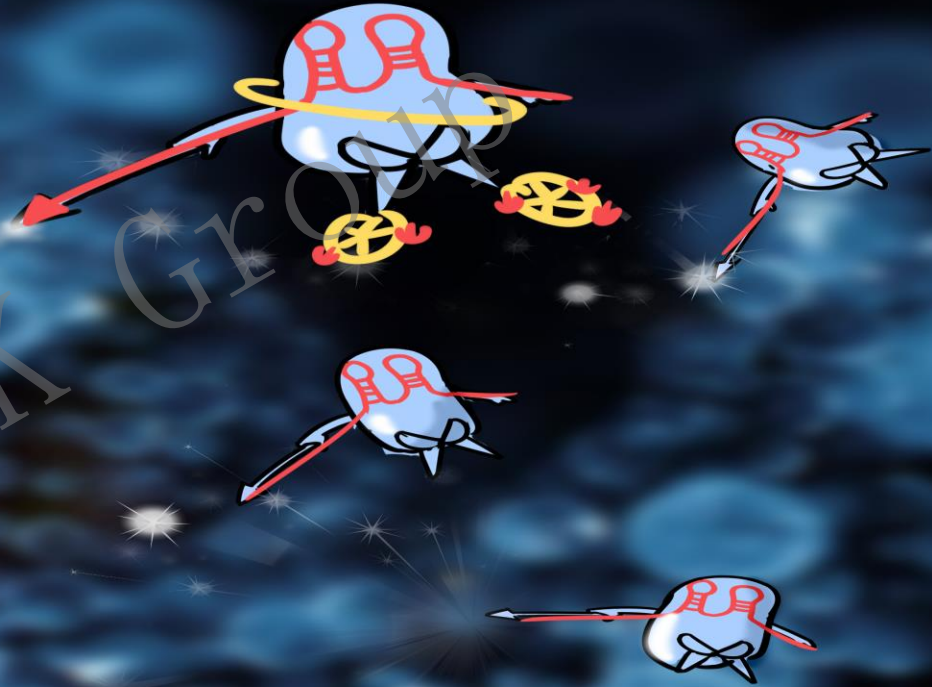
04 双剑合璧

05 老木逢春



sgRNA/Cas9

Mitochondria



Opportunities and Challenges for Mitochondrial Genome Editing, 2018

老木逢春: mitoBEs (mitoDNA base editors)

> Nature. 2020 Jul;583(7817):631-637. doi: 10.1038/s41586-020-2477-4. Epub 2020 Jul 8.

A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing

Beverly Y Mok #^{1 2 3}, Marcos H de Moraes #⁴, Jun Zeng⁴, Dustin E Bosch^{4 5}, Anna V Kotrys^{6 7 8}, Aditya Raguram^{1 2 3}, FoSheng Hsu⁴, Matthew C Radey⁴, S Brook Peterson⁴, Vamsi K Mootha^{6 7}, Joseph D Mougous^{9 10 11}, David R Liu^{12 13 14}

<https://pubmed.ncbi.nlm.nih.gov/32641830/>

> Nat Biotechnol. 2022 Sep;40(9):1378-1387. doi: 10.1038/s41587-022-01256-8. Epub 2022 Apr 4.

CRISPR-free base editors with enhanced activity and expanded targeting scope in mitochondrial and nuclear DNA

Beverly Y Mok^{1 2 3}, Anna V Kotrys^{4 5}, Aditya Raguram^{1 2 3}, Tony P Huang^{1 2 3}, Vamsi K Mootha^{4 5}, David R Liu^{6 7 8}

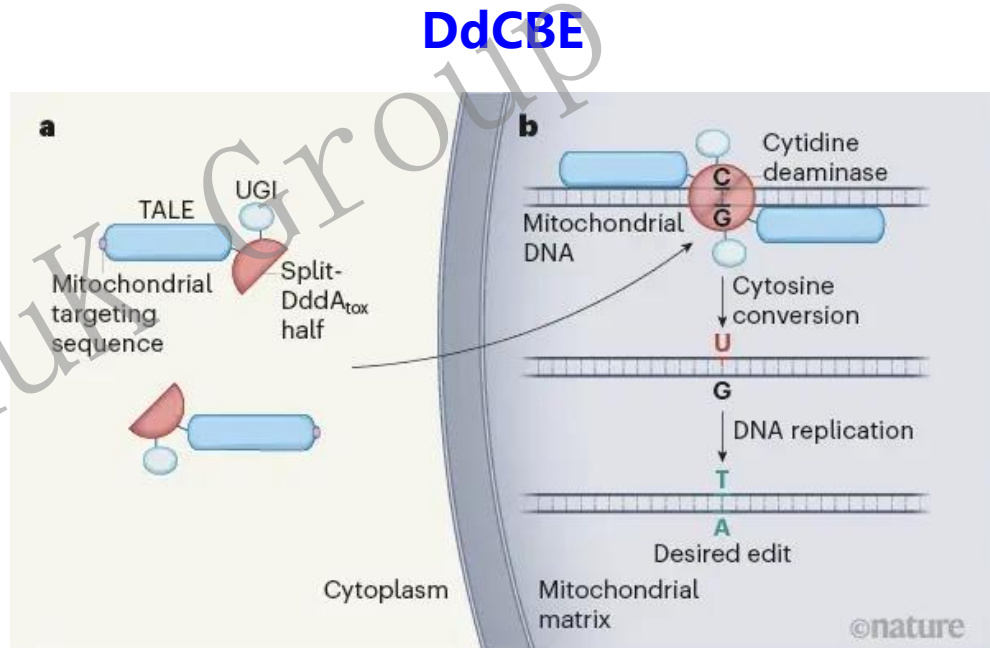
<https://pubmed.ncbi.nlm.nih.gov/35379961/>



David Liu



西北农林科技大学



老木逢春: mitoBEs --TALED

> Cell. 2022 May 12;185(10):1764-1776.e12. doi: 10.1016/j.cell.2022.03.039. Epub 2022 Apr 25.

Targeted A-to-G base editing in human mitochondrial DNA with programmable deaminases

Sung-Ik Cho¹, Seonghyun Lee², Young Geun Mok², Kayeong Lim², Jaesuk Lee¹, Ji Min Lee¹, Eugene Chung¹, Jin-Soo Kim³

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<https://pubmed.ncbi.nlm.nih.gov/35472302/>

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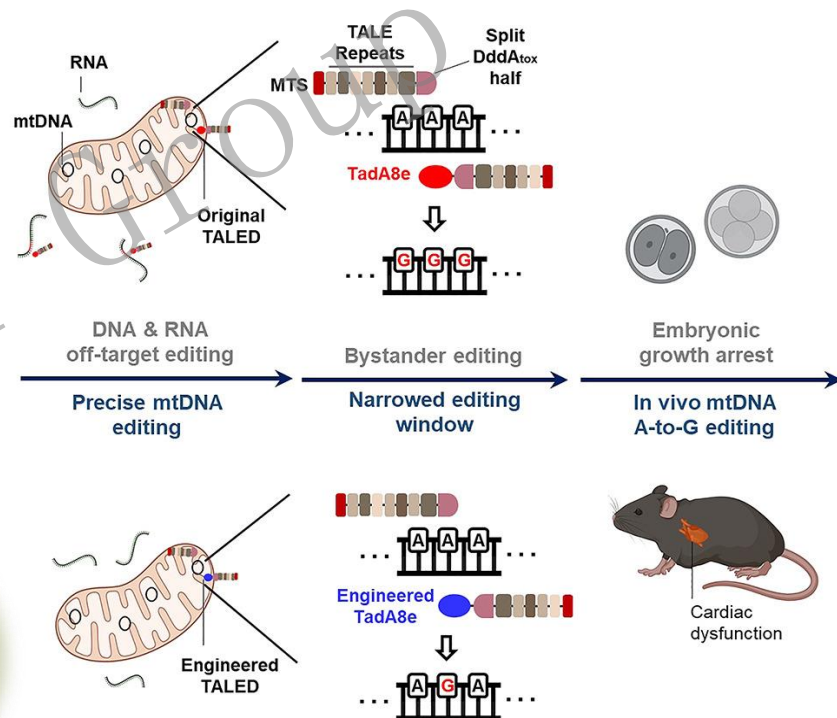
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> Cell. 2024 Jan 4;187(1):95-109.e26. doi: 10.1016/j.cell.2023.11.035.

Engineering TALE-linked deaminases to facilitate precision adenine base editing in mitochondrial DNA

Sung-Ik Cho¹, Kayeong Lim², Seongho Hong³, Jaesuk Lee⁴, Annie Kim⁵, Chae Jin Lim⁶, Seungmin Ryou⁶, Ji Min Lee⁴, Young Geun Mok⁷, Eugene Chung⁵, Sanghun Kim⁸, Seunghun Han⁹, Sang-Mi Cho¹⁰, Jieun Kim³, Eun-Kyoung Kim¹⁰, Ki-Hoan Nam¹⁰, Yeji Cho¹⁰, Minkyung Choi⁵, Tae Hyeon An¹¹, Kyoung-Jin Oh¹¹, Seonghyun Lee¹², Hyunji Lee¹³, Jin-Soo Kim¹⁴

韩国Jin-Soo Kim (金镇洙)



老木逢春: mitoBEs

> Nat Biotechnol. 2024 Mar;42(3):498-509. doi: 10.1038/s41587-023-01791-y. Epub 2023 May 22.

Strand-selective base editing of human mitochondrial DNA using mitoBEs

Zongyi Yi ^{#1,2}, Xiaoxue Zhang ^{#1,3}, Wei Tang ^{1,3}, Ying Yu ¹, Xiaoxu Wei ^{1,3}, Xue Zhang ², Wensheng Wei ^{4,5}

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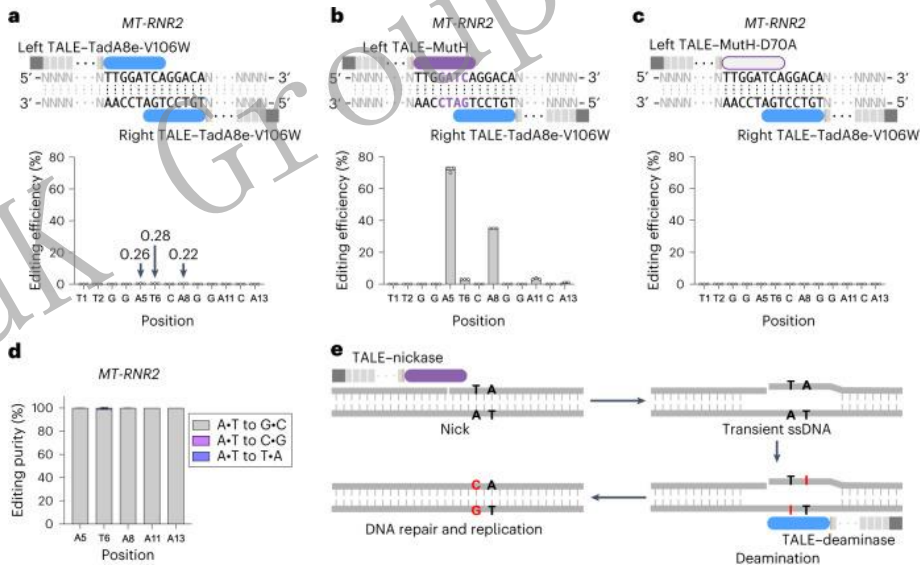
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> Nat Commun. 2023 Feb 16;14(1):874. doi: 10.1038/s41467-023-36600-2.

DddA homolog search and engineering expand sequence compatibility of mitochondrial base editing

Li Mi ^{# 1}, Ming Shi ^{# 1 2}, Yu-Xuan Li ¹, Gang Xie ³, Xichen Rao ^{4 5}, Damu Wu ⁴, Aimin Cheng ², Mengxiao Niu ¹, Fengli Xu ¹, Ying Yu ^{4 5 6 7 8}, Ning Gao ^{2 4}, Wensheng Wei ^{4 5 6 7 8}, Xianhua Wang ¹, Yangming Wang ⁹

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北京大学汪阳明

> Adv Sci (Weinh). 2024 Jan;11(3):e2304113. doi: 10.1002/adv.202304113. Epub 2023 Nov 20.

Enhanced C-To-T and A-To-G Base Editing in Mitochondrial DNA with Engineered DdCBE and TALED

Yinghui Wei ^{1 2}, Ming Jin ³, Shuhong Huang ¹, Fangyao Yao ¹, Ningxin Ren ⁴, Kun Xu ¹, Shangpu Li ¹, Pengfei Gao ¹, Yingsi Zhou ⁴, Yulin Chen ^{1 2}, Hui Yang ^{4 5}, Wen Li ⁶, Chunlong Xu ⁵, Meiling Zhang ⁶, Xiaolong Wang ^{1 2}

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老木逢春: cpBEs (chloroplast DNA base editors)

> Nat Plants. 2022 Dec;8(12):1378-1384. doi: 10.1038/s41477-022-01279-8. Epub 2022 Dec 1.

Targeted A-to-G base editing of chloroplast DNA in plants

Young Geun Mok ^{# 1 2}, Sunghyun Hong ^{# 1 2}, Su-Ji Bae ¹, Sung-Ik Cho ^{1 3}, Jin-Soo Kim ^{4 5}

Affiliations + expand

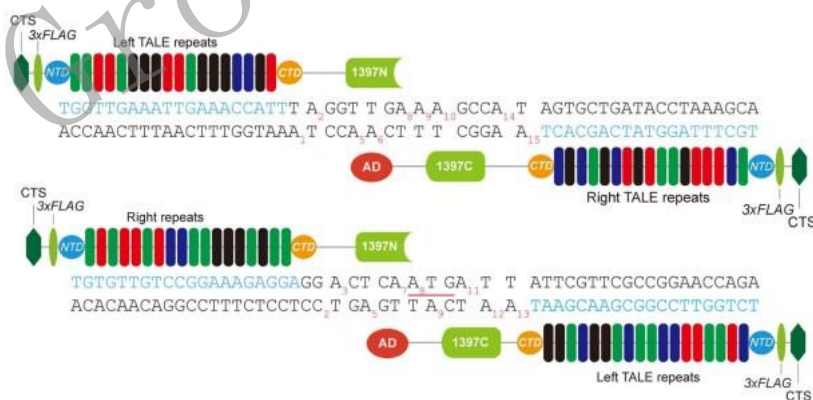
PMID: 36456803 PMCID: PMC9788985 DOI: 10.1038/s41477-022-01279-8

<https://pubmed.ncbi.nlm.nih.gov/36456803/>



韩国Jin-Soo Kim (金镇洙)

Ls psbA



西北农林科技大学

老木逢春: **cpBEs** (cpDNA base editors)

Herbicide-resistant plants produced by precision adenine base editing in plastid DNA.

Mok YG, Hong S, Seo DI, Choi S, Kim HK, Jin DM, Lee JJ, Kim JS.

Nat Plants. 2024 Sep 26. doi: 10.1038/s41477-024-01808-7. Online ahead of print.

PMID: 39327461

Broad range plastid genome editing with monomeric TALE-linked cytosine and dual base editors.

Wang X, Fang T, Lu J, Tripathi L, Qi Y.

Plant Biotechnol J. 2024 Sep;22(9):2441-2443. doi: 10.1111/pbi.14358. Epub 2024 May 6.

PMID: 38709858 [Free PMC article.](#) No abstract available.

Targeted C•G-to-T•A base editing with TALE-cytosine deaminases in plants.

Zhang D, Pries V, Boch J.

BMC Biol. 2024 Apr 29;22(1):99. doi: 10.1186/s12915-024-01895-0.

PMID: 38679734 [Free PMC article.](#)



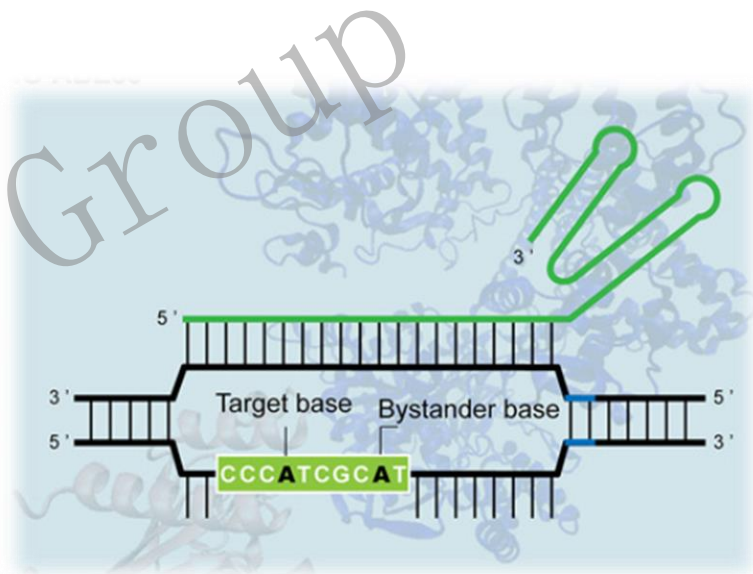
拓展思考3:

精准的碱基编辑有什么意义?

BEs存在问题: 旁编辑, gRNA依赖性

脱靶, gRNA非依赖性脱靶?

如何利用BEs进行基因“敲除”?





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