生物育种专业·基因编辑技术课程

第三章：补天之石—基于DSB的基因编辑策略

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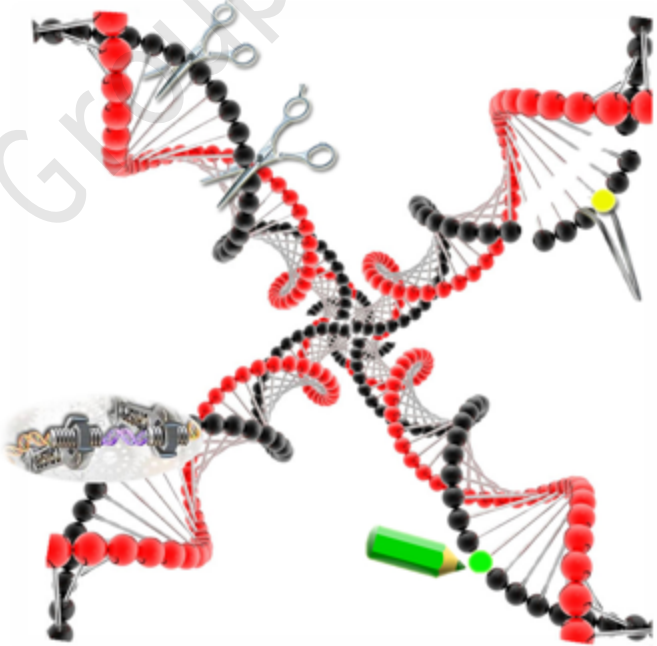
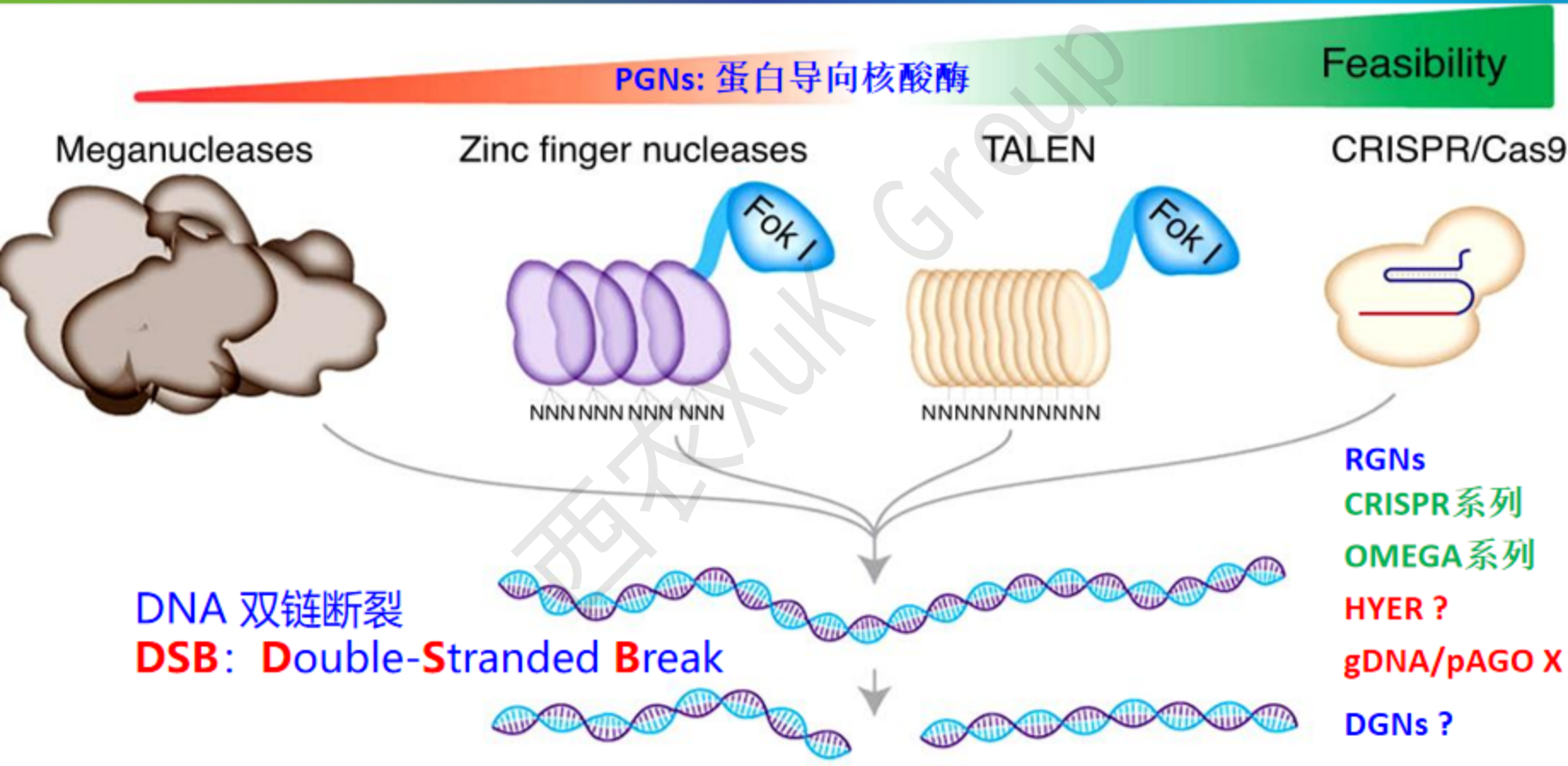
上节课拓展思考：

什么是基因编辑？

如何利用上述工具实现基因编辑？



基因剪刀手—人工特异性核酸酶技术



DNA断裂

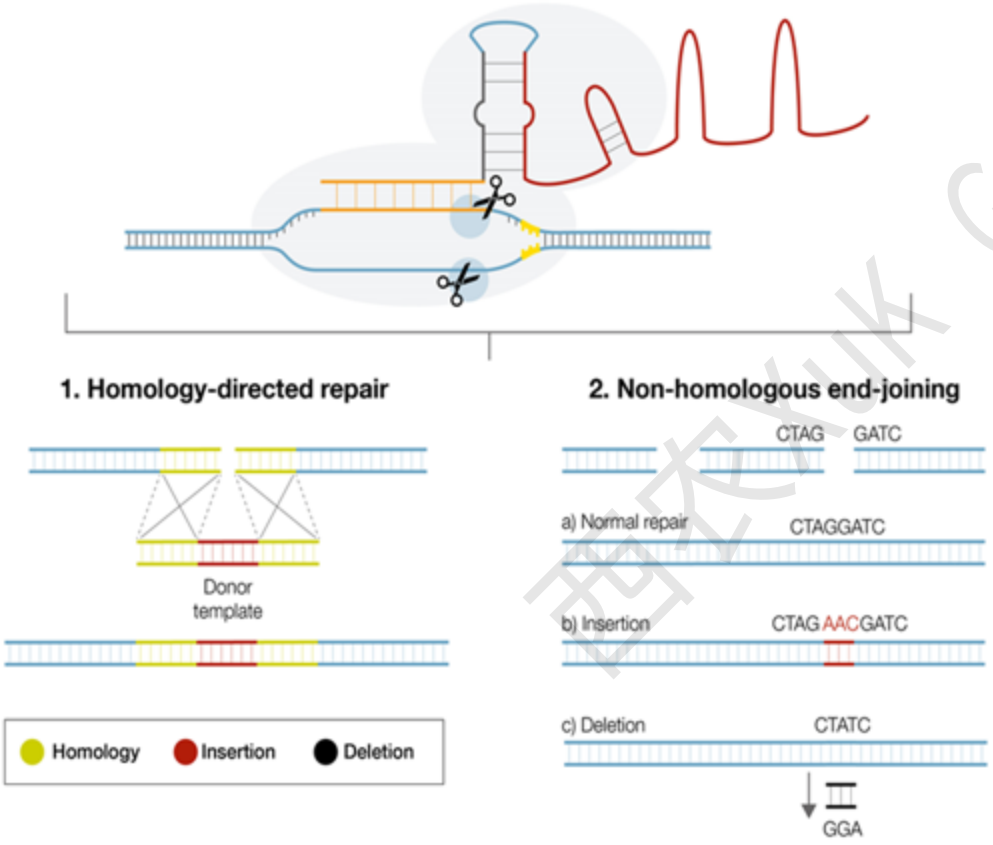
对于细胞来说

就是天塌下来的灾难！

细胞的小宇宙

也有自己的“女娲”

补天之“石”—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

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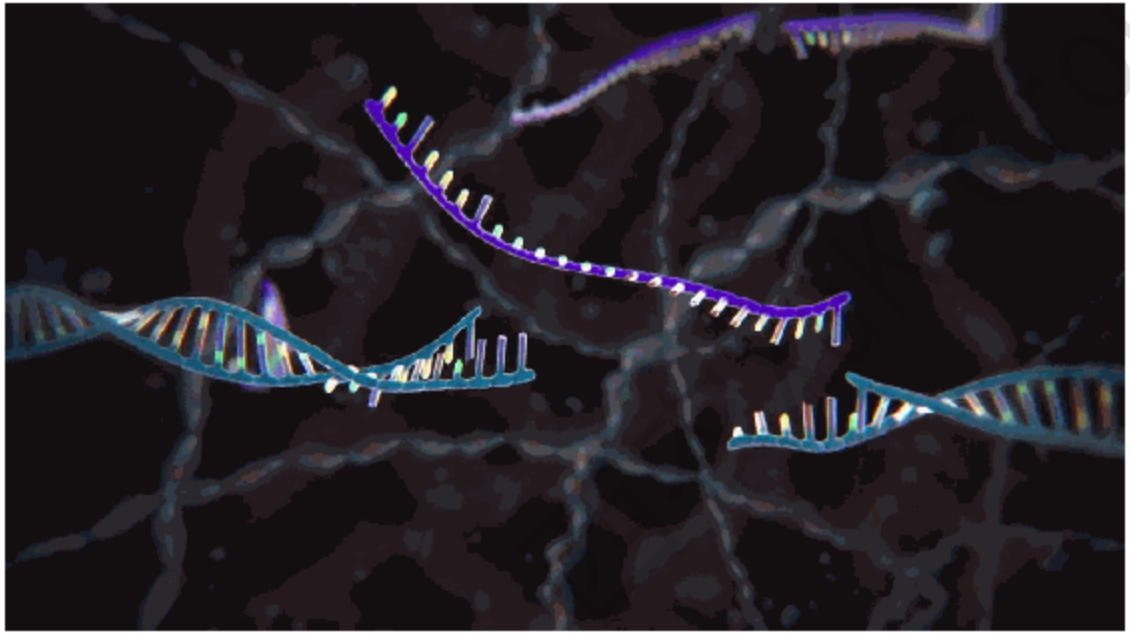
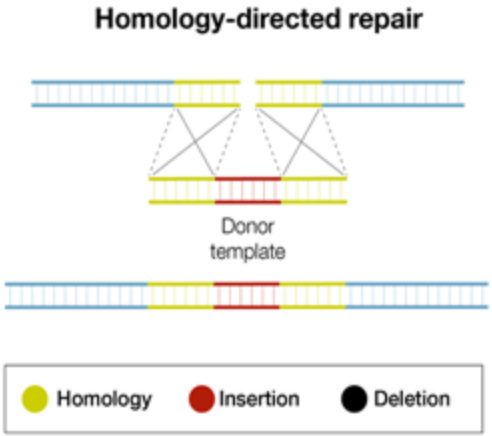
利用HDR进行基因编辑（敲入/替换/点编辑）





HDR→KI/Replace

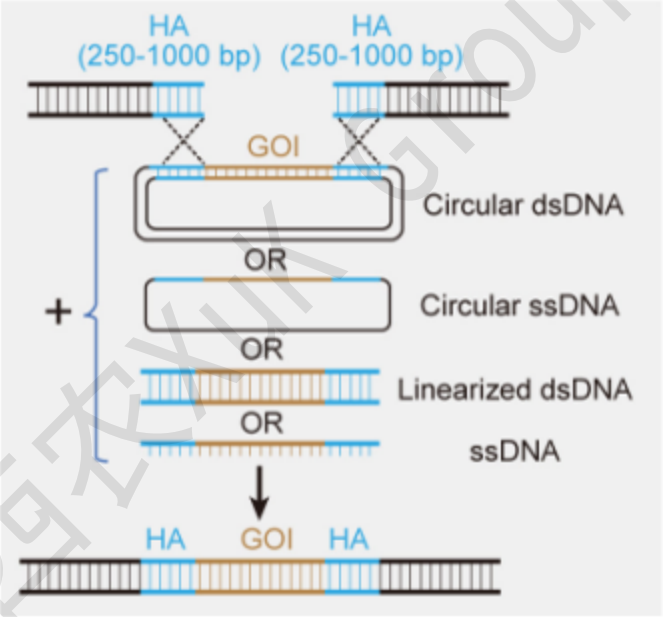
HDR→Point/Base editing

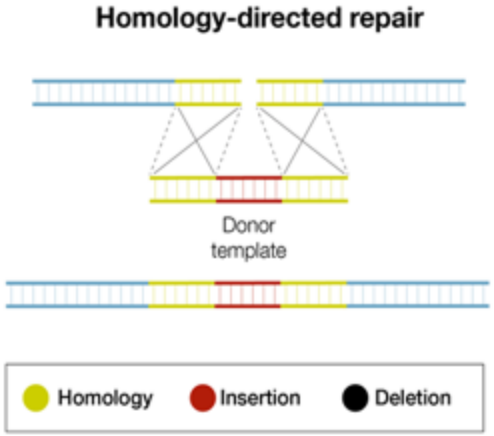
利用HDR进行基因编辑（敲入/替换/点编辑）

HR or **HRR**:
Homologous recombination
同源重组
基因打靶--打靶载体

TILD:
Targeted **I**ntegration with **L**inearized dsDNA **D**onor

SSTR:
Single-**S**trand **T**emplated DNA **R**epair

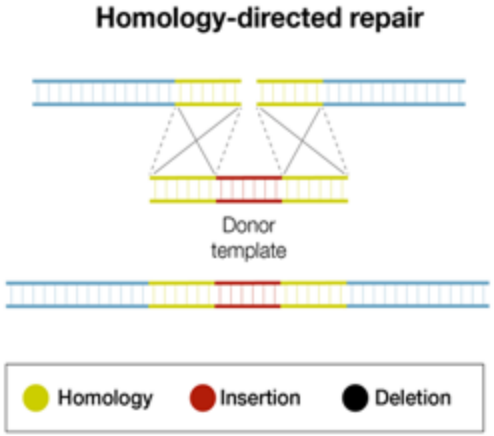




HDR→KI/Replace

HDR→Point/Base editing

利用HDR进行基因编辑（敲入/替换/点编辑）



HDR→KI/Replace

HDR→Point/Base editing

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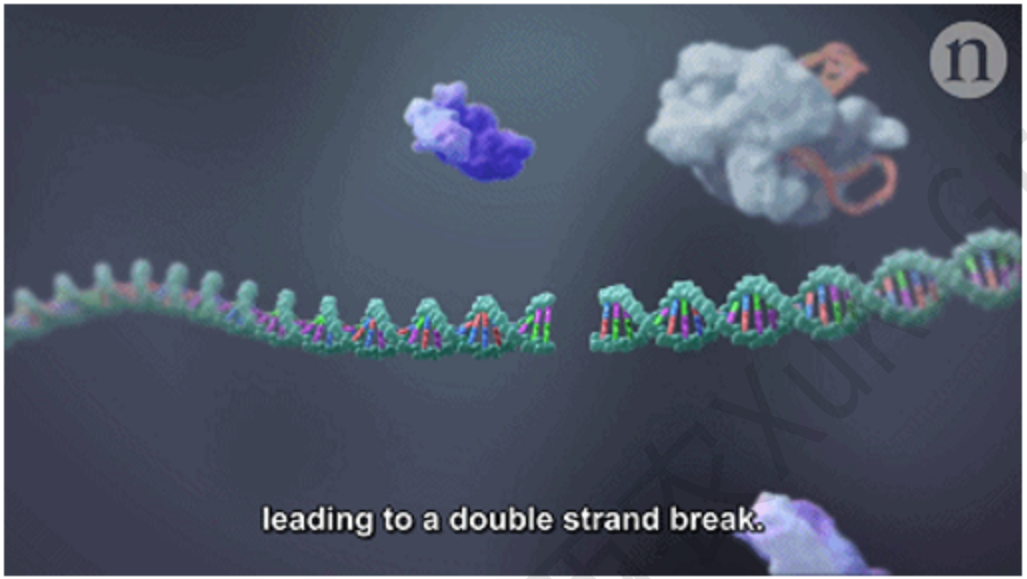
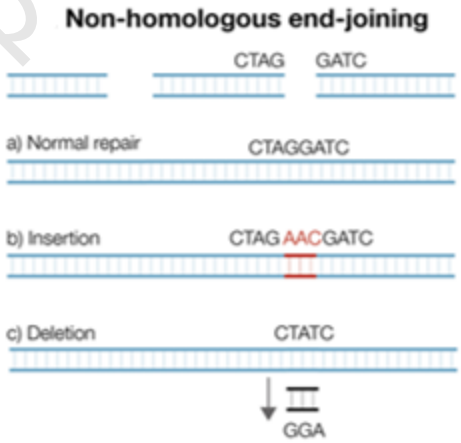
利用NHEJ进行基因敲除



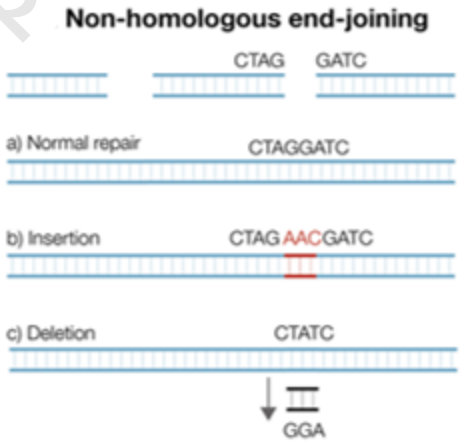


NHEJ→Indels→KO

NHEJ→Fragment deletion

NHEJ→插入或缺失（indels）→移码突变/片段性删除

利用NHEJ进行基因敲除



NHEJ→Indels→KO

NHEJ→Fragment deletion

cNHEJ vs aNHEJ

https://pubmed.ncbi.nlm.nih.gov/30033371/

cNHEJ:
canonic NHEJ、classical NHEJ（cNHEJ）

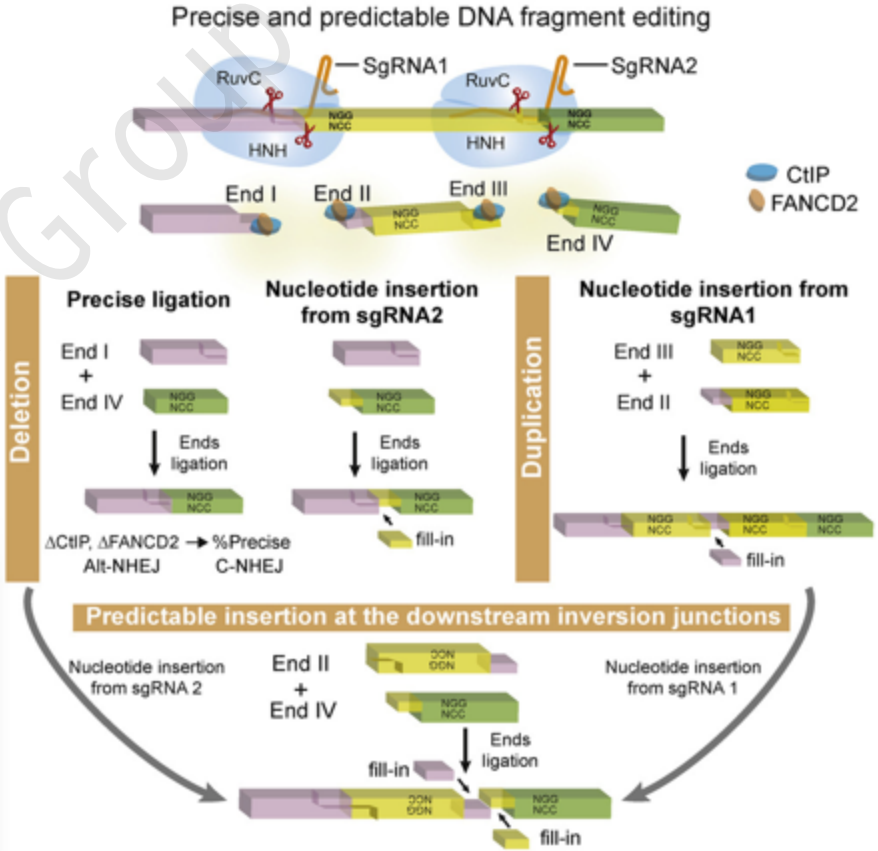
aNHEJ/alt-NHEJ
alternative NHEJ
Microhomology-Mediated End Joining (MMEJ)

Mol Cell. 2018 Aug 16;71(4):498-509.e4. doi: 10.1016/j.molcel.2018.06.021. Epub 2018 Jul 19.

Precise and Predictable CRISPR Chromosomal Rearrangements Reveal Principles of Cas9-Mediated Nucleotide Insertion

Jia Shou [1], Jinhuan Li [1], Yingbin Liu [2], Qiang Wu [3]

上海交通大学 吴强



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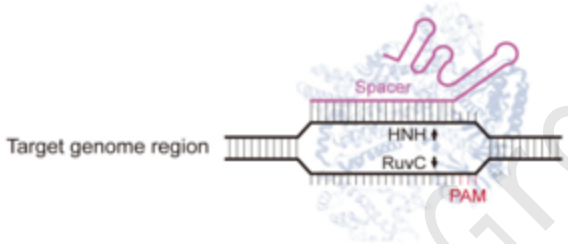
玩转供体DNA

https://pubmed.ncbi.nlm.nih.gov/38379727/

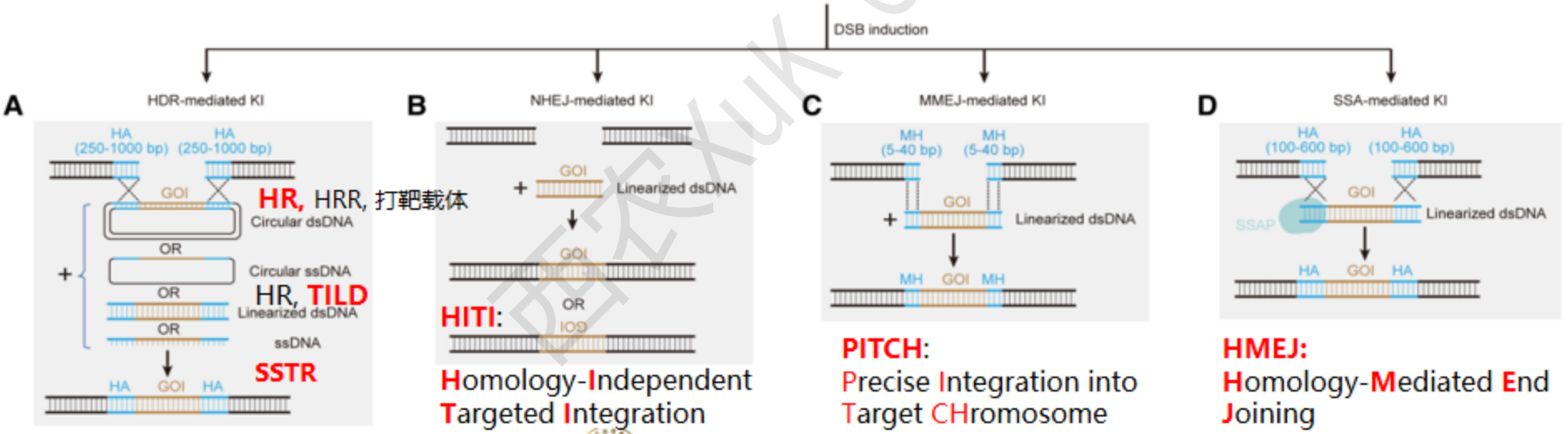
Donor: 供体



Review > Mol Ther Nucleic Acids. 2024 Feb 5;35(1):102138. doi: 10.1016/j.omtn.2024.102138. eCollection 2024 Mar 12.

Recent advances in CRISPR-Cas9-based genome insertion technologies

Xinwen Chen [1,2], Jingjing Du [1,2], Shaowei Yun [1,2], Chaoyou Xue [3,4], Yao Yao [1,2], Shuquan Rao [1,2]



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国内学者多有相关报道

玩转供体DNA

https://pubmed.ncbi.nlm.nih.gov/38379727/

Review > Mol Ther Nucleic Acids. 2024 Feb 5;35(1):102138. doi: 10.1016/j.omtn.2024.102138. eCollection 2024 Mar 12.

Recent advances in CRISPR-Cas9-based genome insertion technologies

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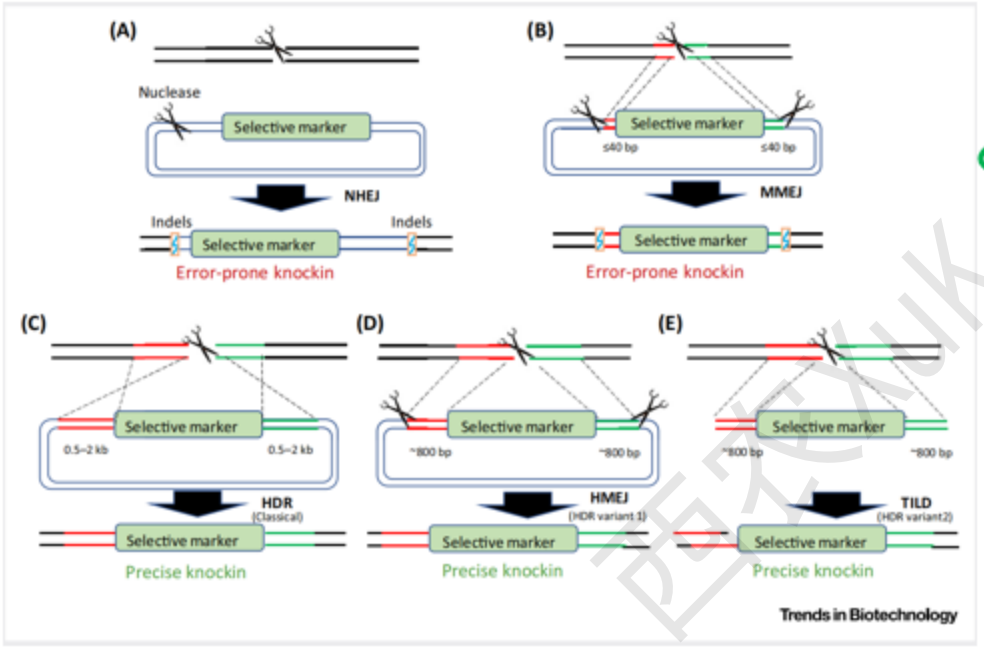
Table 1. Comparison of technologies of targeted genome insertion mentioned in this review

Types of knock-in technologies	Genome insertion component	Homology arms	Capability of knock-in	Cell cycle dependence	DSB generation	Genotoxicity	Undesired outcome	Efficiency in mammalian cells
DSB repair-dependent technology								
HRR-mediated knock-in	programmable nuclease (ZFN, TALEN or CRISPR-Cas), donor template (dsDNA or ssDNA)	50–1,000 bp (even longer)	1–10 kb	limited to S/G2 phase	yes	DSB-associated genotoxicity	modest indels and off-target editing[a]	high efficiency
SSA-mediated knock-in	Programmable nuclease (CRISPR-Cas), linearized donor template (dsDNA)	100–600 bp	~2 kb	limited to S/G2 phase	yes[b]	DSB-associated cellular toxicity	relatively low indel and low off-target editing	modest efficiency
NHEJ-mediated knock-in	programmable nuclease (ZFN, TALEN or CRISPR-Cas), linearized donor template (dsDNA)	None	>1 kb	No	Yes	DSB-associated genotoxicity	high indels, inversion of donor DNA, duplication and off-target editing	high efficiency
MMEJ-mediated knock-in	programmable nuclease (ZFN, TALEN or CRISPR-Cas), linearized donor template (dsDNA)	5–40 bp	≤5 kb	G1/early S phase	yes	DSB-associated genotoxicity	high indels and low off-target editing	modest efficiency

玩转供体DNA

https://pubmed.ncbi.nlm.nih.gov/30135027/



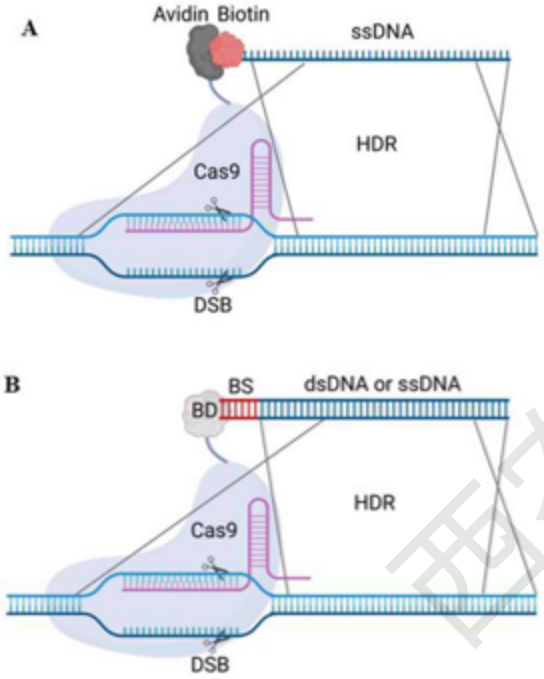
Review > Trends Biotechnol. 2019 Jan;37(1):56-71. doi: 10.1016/j.tibtech.2018.07.017. Epub 2018 Aug 19.

Strategies for the Enrichment and Selection of Genetically Modified Cells

Chonghua Ren [1], Kun Xu [2], David Jay Segal [3], Zhiying Zhang [4]

HDR: ?
HR: TLR
SSA: HMEJ
MMEJ: PITCH
NHEJ: HITI

DAS: Donor-Adapting System



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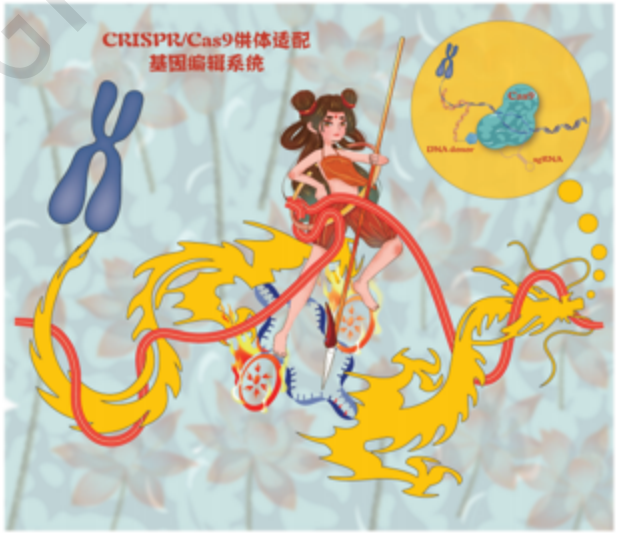

西北农林科技大学 https://lab.gewu.pro/groups/T8B5U1

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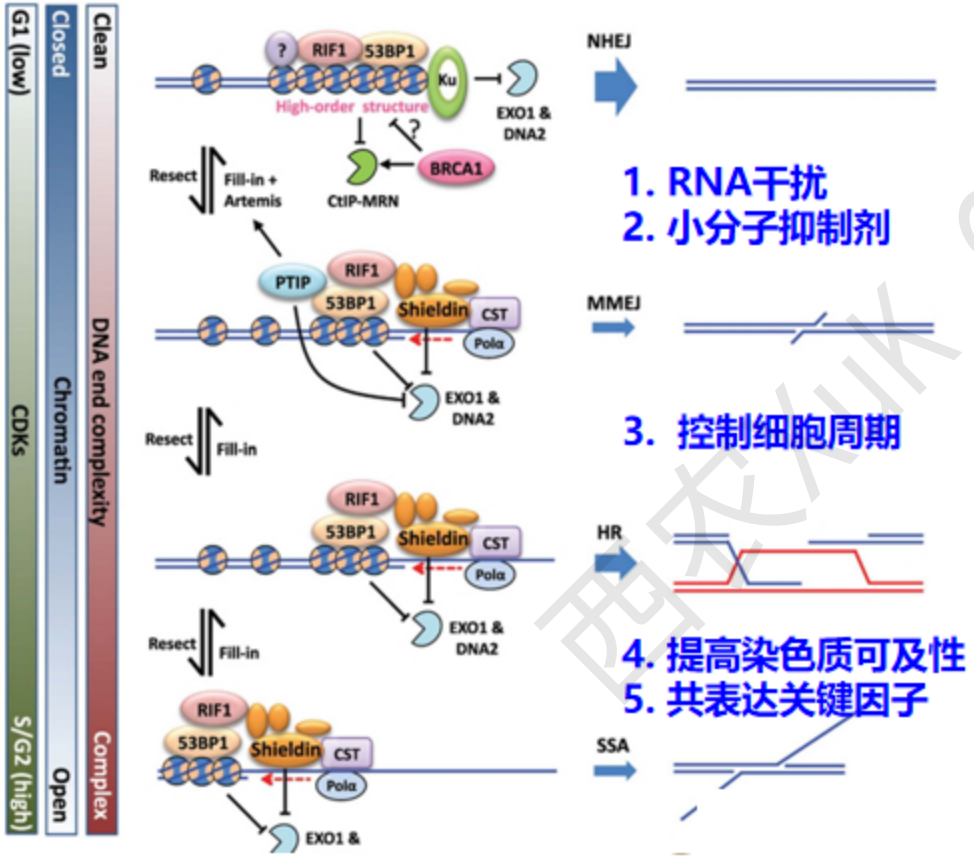
拔赵易汉：偷换取胜

信所出奇兵二千骑，共候赵空壁逐利，则**驰入赵壁，皆拔赵旗，立汉赤帜二千**。赵军已不胜，不能得信等，欲还归壁，壁皆汉赤帜，**而大惊，以为汉皆已得赵王将矣，兵遂乱**，遁走，赵将虽斩之，不能禁也。于是汉兵夹击，**大破虏赵军。**

DSB修复机制的竞争及提高HR/HDR效率策略



1. RNA干扰
2. 小分子抑制剂

3. 控制细胞周期

4. 提高染色质可及性
5. 共表达关键因子

Review > Essays Biochem. 2020 Oct 26;64(5):765-777. doi: 10.1042/EBC20200007.

Repair pathway choice for double-strand breaks

Yixi Xu [1], Dongyi Xu [1]

https://pubmed.ncbi.nlm.nih.gov/30135027/

> Nat Methods. 2023 Sep;20(9):1388-1399. doi: 10.1038/s41592-023-01949-1. Epub 2023 Jul 20.

Efficient high-precision homology-directed repair-dependent genome editing by HDRobust

Stephan Riesenberg [1], Philipp Kanis [2], Dominik Macak [2], Damian Wollny [2], Dorothee Düsterhöft [2], Johannes Kowalewski [2], Nelly Helmbrecht [2], Tomislav Maricic [2], Svante Pääbo [2 3]

https://pubmed.ncbi.nlm.nih.gov/37474806/

Enhancing CRISPR/Cas9-mediated homology-directed repair in mammalian cells by expressing Saccharomyces cerevisiae Rad52

S Shao, C Ren, Z Liu, Y Bai, Z Chen, Z Wei, X Wang, Z Zhang, K Xu

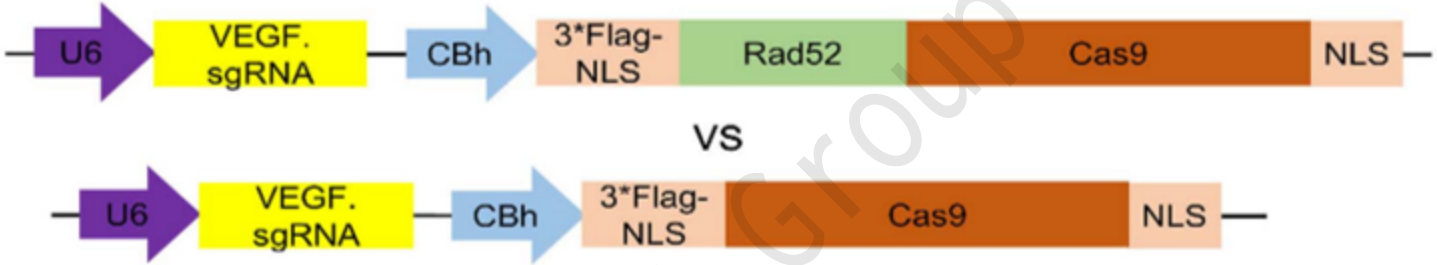
The international journal of biochemistry & cell biology 92, 43-52

104 2017

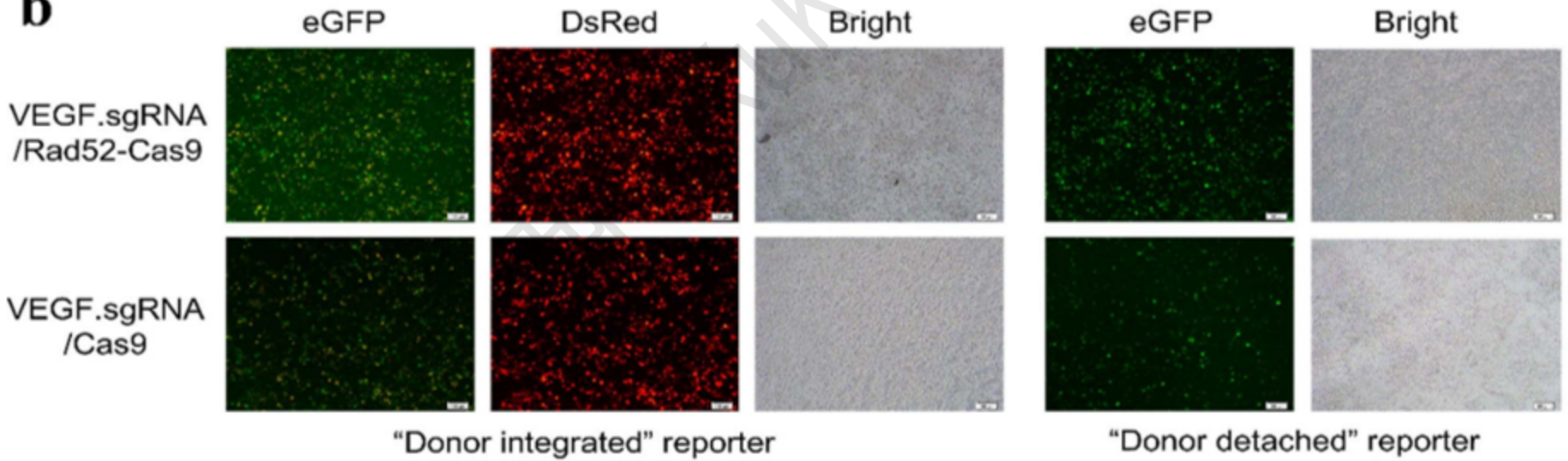
https://lab.gewu.pro/groups/lunwen/T8B5U1

将Rad52与Cas9共表达能显著提高HDR效率

a



b



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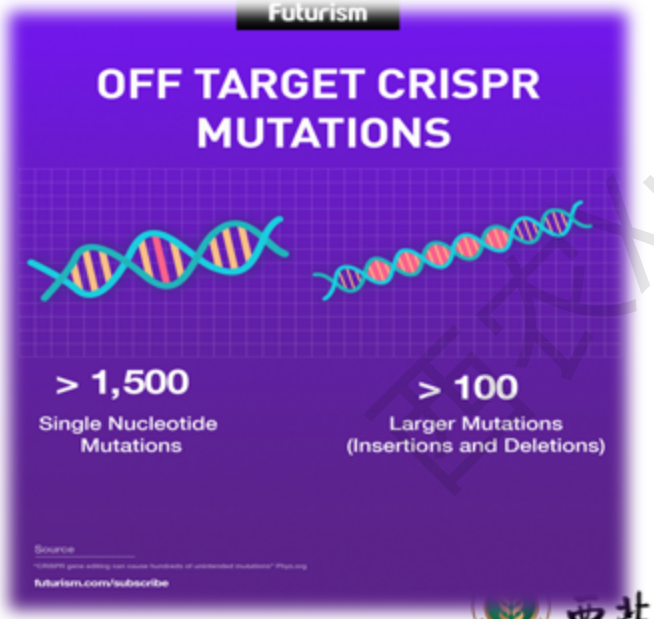
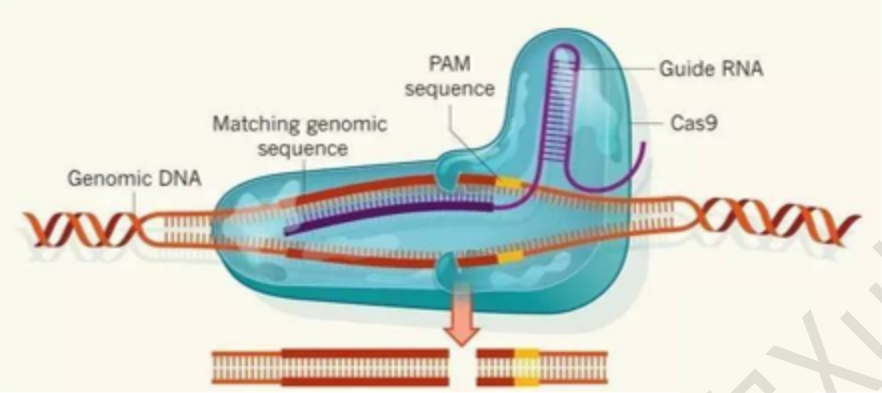
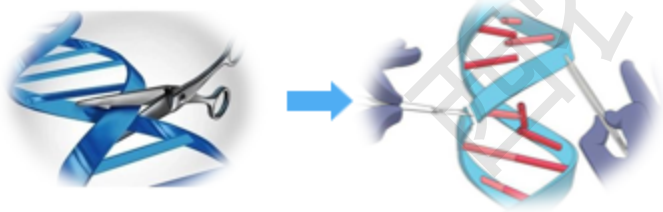
脱靶效应：off-target

基因编辑过程中，特异性核酸酶（CRISPR/Cas9“剪刀”）在靶序列之外的位点切割，造成意外的基因突变的现象。



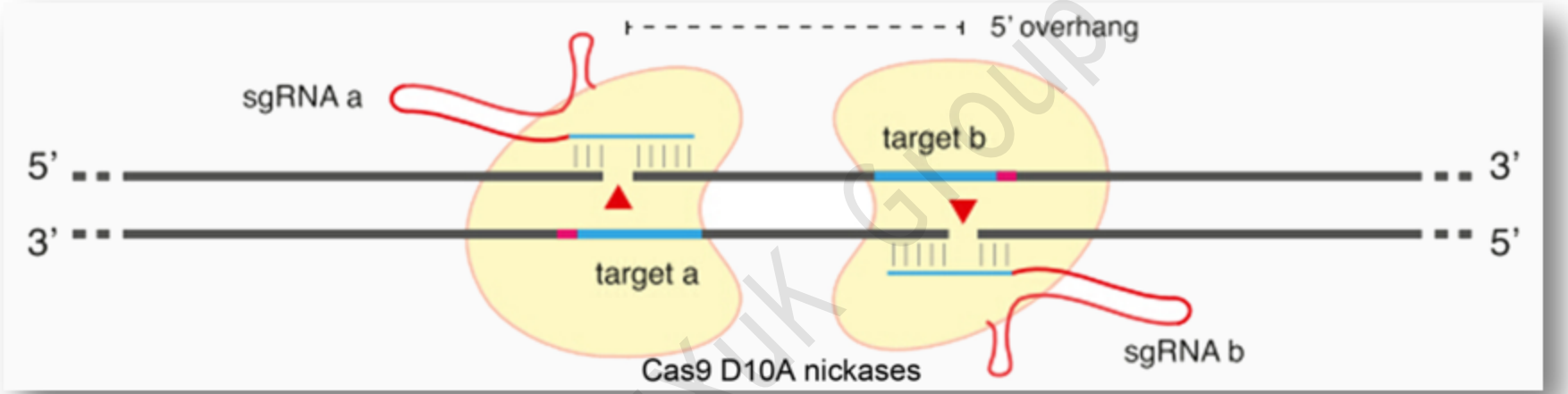
坏掉的剪刀—“nCas9”和“dCas9”





- **以CRISPR/Cas9为例**
- **Cas9缺口酶**（nCas9，Cas9n）：
  D10A or H840A
- **“死掉”的Cas9** (dead Cas9, dCas9):
  D10A and H840A

nCas9介导的基因编辑



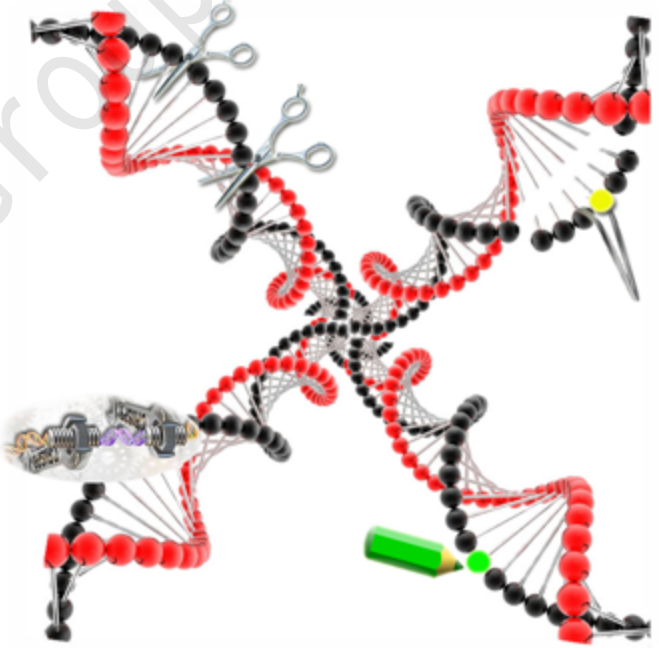
- 双sgRNA/nCas9
- HDR→KI/Point Editing
- 降低脱靶效应

拓展思考2：

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?



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