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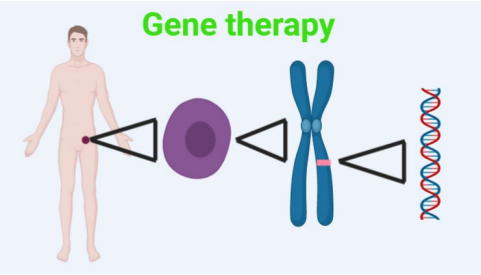
CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement

Key words: Genome editing; CRISPR/Cas; COVID-19; Cancer; Precision breeding; Crop improvement; Gene knockout/in; Gene repair/replacement

Research Summary

This review focuses on CRISPR/Cas genome editing technology, one of the hottest biotechnological techniques, which won the Nobel Prize in 2020. Three major topics are covered in this review.

- Principles and types of CRISPR/Cas genome editors
- CRISPR/Cas-based gene therapy
- CRISPR/Cas-based crop improvement



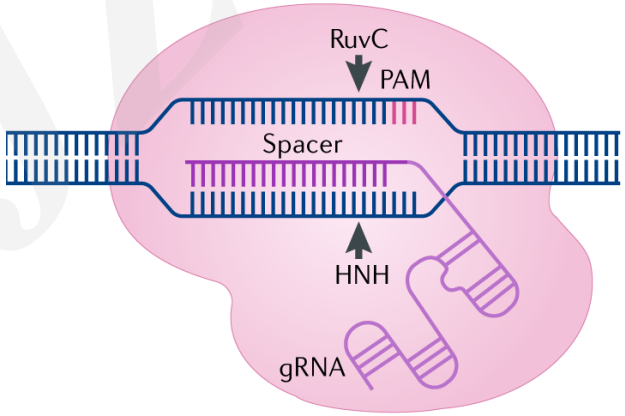


Table 1. Comparison of the major genome editing tools

	Meganucleases	ZFN	TALEN	CRISPR/Cas
Interaction	Protein-DNA	Protein-DNA	Protein-DNA	DNA-DNA or DNA-RNA
Recognition site	Large (12-40 bp dsDNA)	Long	Long	short
Required agents		ZFN with a FokI DNA cleaving domain and a DNAS binding domain	TALEN with a FokI DNA cleaving domain and a DNAS binding domain (TAL repeats)	Cas and sgRNA
Required PAM	No	No	No	Yes
Inducing double-strand break?	Yes	Yes	Yes	Yes
Cell toxicity	Low	Low	Low	High
Specificity	Very high	Very high	Very high	High
Off-target	Low	Low	Low	Relatively high
Multiplex?	Difficult	Difficult	Difficult	Yes
Editing efficiency	Low	Relatively low	Relatively low	High
Single nucleic acid targeting	Yes	Yes	Yes	Yes
Cost	High	High	High	Low
Easy for construction?	No	Relatively hard	Relatively hard	Simple, easy, and robust

Figure 4

Table 2. Comparison of major CRISPR/Cas genome/gene editors

	Regular CRISPR/Cas editor without DNA template	Regular CRISPR/Cas editor with DNA template	CRISPR/Cas epigenetic editor	CRISPRi editor	CRISPRa editor	Base editor	Prime editor
Cas enzyme	Cas	Cas	dCas	dCas	dCas	dCas	nCas
Additional enzymes	-	-	Epigenetic modifier, including DNA methyltransferase	dCas alone or fusing with a repressor	Fusing an effector with dCas9	Nucleobase deaminase enzyme	Reverse transcriptase
Induced DSB or SSB	DSB	DSB	No	No	No	No	SSB
Require DNA template?	No	Yes	No	No	No	No	No
gRNA	sgRNA	sgRNA	sgRNA	sgRNA	sgRNA	sgRNA	pegRNA
Major application	Gene knockout, inducing silence mutations	Gene knockin, DNA replacement	Regulation of epigenome and gene expression	Knock down	Activation of gene expression	Base change	Sequence repair

Table 4. Improvement of crop yield and associated traits by using CRISPR/Cas

Crop species	Targeted gene	Improved trait	CRISPR/Cas editor	References
Rice	gn1a, dep1 and gs3	Grain number, dense erect panicles, and larger grain size	CRISPR/Cas9	(Li, et al., 2016a)
Rice	gw2, gw5 and tgw6	Grain size and crop yield	CRISPR/Cas9	(Xu, et al., 2016)
Rice	OsPAO5	Grain weight, grain numbers and yield	CRISPR/Cas9	(Lv, et al., 2020)
Switchgrass	Tb1	Tillers and fresh biomass	CRISPR/Cas9	(Liu, et al., 2020b)
Rapeseed	BnaMAX1	Plant architecture and yield	CRISPR/Cas9	(Zheng, et al., 2020)
Soybean	GmLHY	Plant height and internode length	CRISPR/Cas9	(Cheng et al., 2019)
Soybean	AP1	Flowering time and plant height	CRISPR/Cas9	(Chen et al., 2020b)
Soybean	GmNMHC5	Flowering and maturity	CRISPR/Cas9	(Wang et al., 2020b)
Wheat	TaGW2	Grain weight and protein content	CRISPR/Cas9	(Zhang, et al., 2018c)
Rice	OsGcs1	High-quality sugar production	CRISPR/Cas9	(Honma, et al., 2020)
Rice	Ehd1	Basic vegetative growth	CRISPR/Cas9	(Wu, et al., 2020)