PRIME EDITING

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GEFSES-CREATE

Review

Article

Search-and-replace genome editing without double-strand breaks or donor DNA

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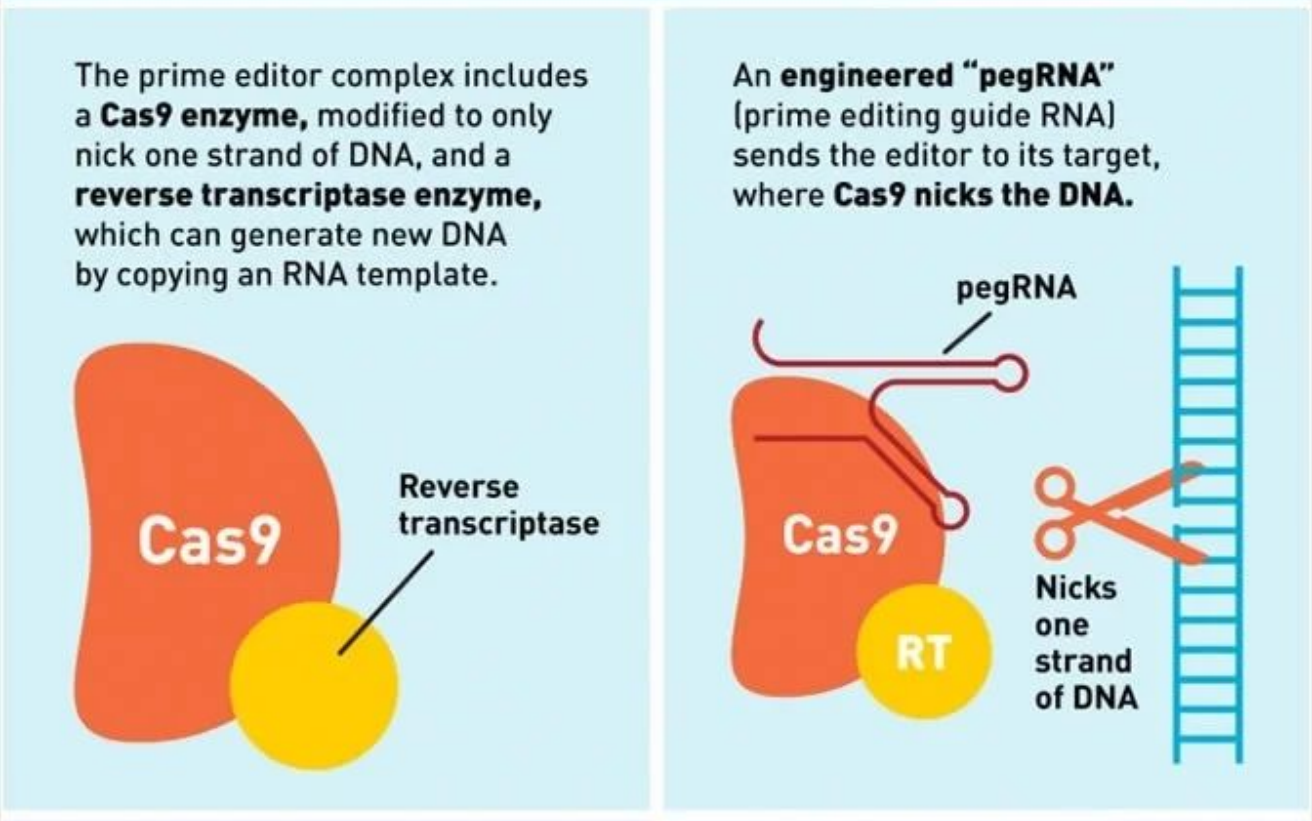
Most genetic variants that contribute to disease[1] are challenging to correct efficiently and without excess byproducts[2–5]. Here we describe prime editing, a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 endonuclease fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. We

- All 4 transitions, all 8 transversions
- Insertions (1 to 44 bp)
- Deletions (1 to 80 bp)
- Combinations of above

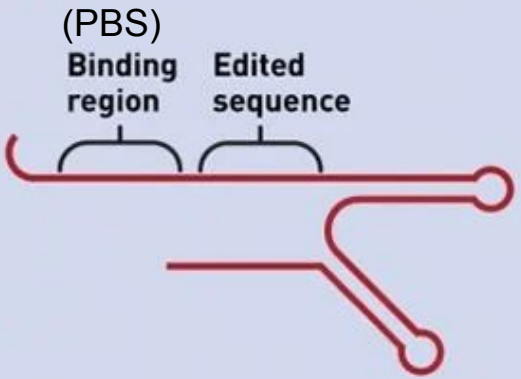
Prime Editor



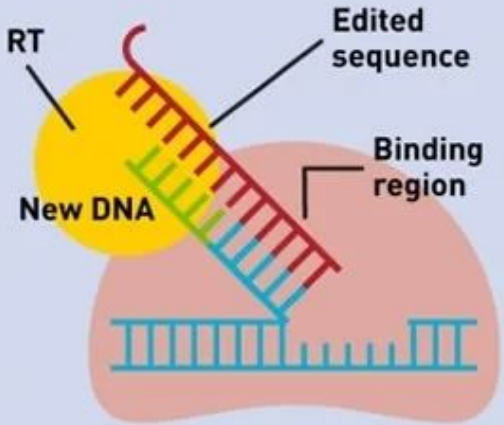
https://www.broadinstitute.org/visuals/prime-editing-search-and-replace-genome-editing

pegRNA

The **pegRNA** has two special components: **a section that binds to the nicked DNA,** preparing the nicked strand to have new DNA letters added, and a **section of RNA letters** that encode the desired edit.



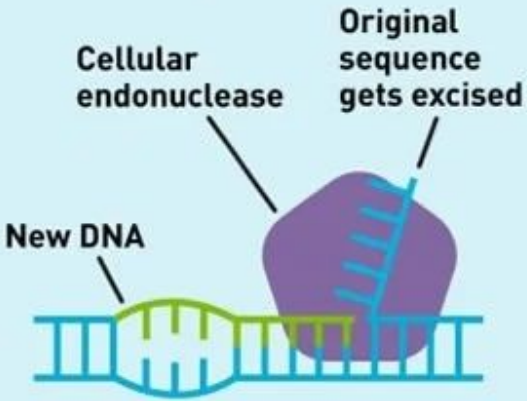
To transfer the edited sequence from the pegRNA to the target DNA, the **reverse transcriptase** reads the **RNA** and attaches the corresponding DNA letters to the end of the **nicked DNA.**



https://www.broadinstitute.org/visuals/prime-editing-search-and-replace-genome-editing

Prime Editing

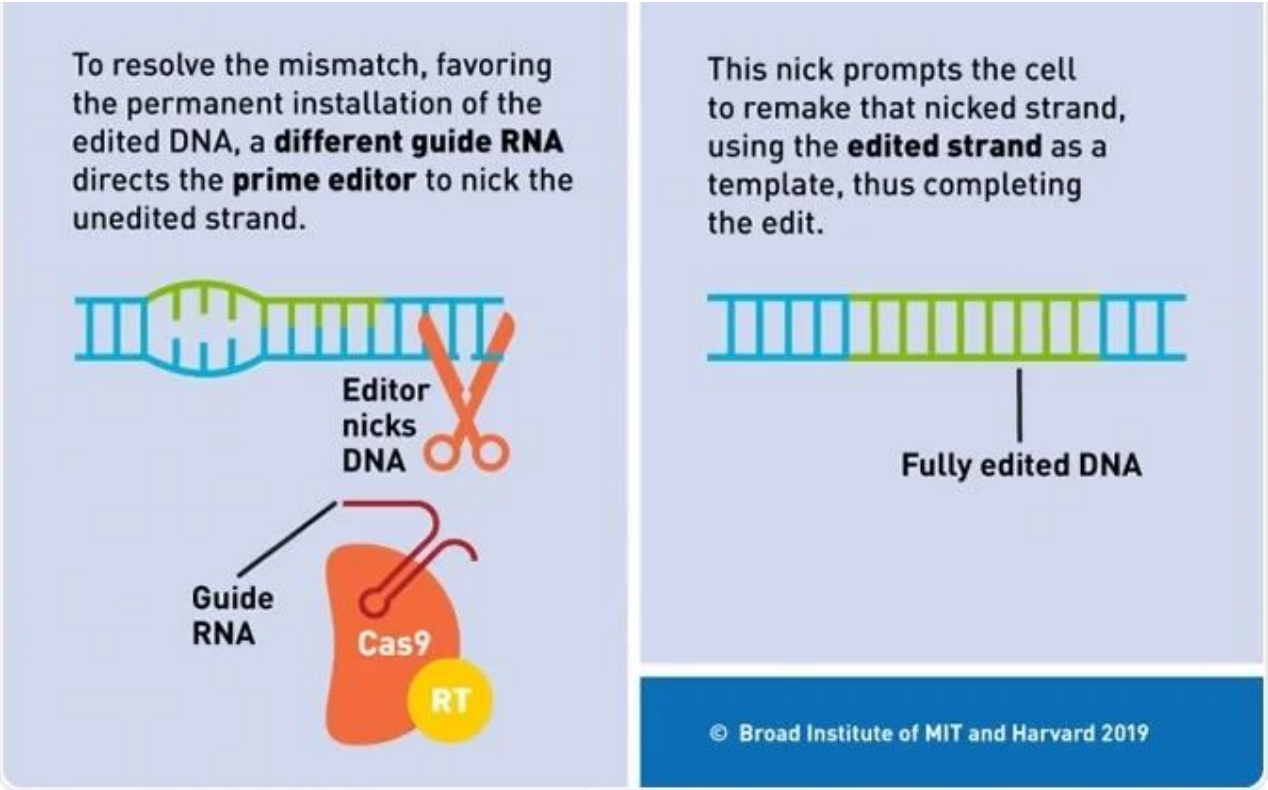
An **endonuclease** in the cell naturally excises the **old segment** of DNA and seals the **new letters** into the genome.



Now, the target site is left with **one edited strand and one unedited strand.**

https://www.broadinstitute.org/visuals/prime-editing-search-and-replace-genome-editing

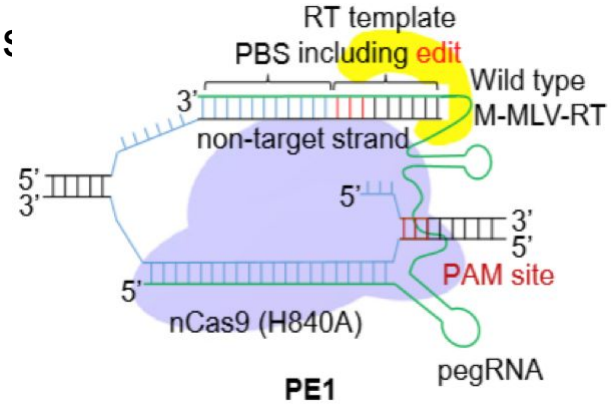
Prime Editing (cont.)



https://www.broadinstitute.org/visuals/prime-editing-search-and-replace-genome-editing

First Generation of Prime Editors (PE 1)

- Fused an M-MLV (Moloney murine leukemia virus RT to the C terminus of Cas9 nickase
- Used different primer binding system (PBS) lengths: 8-15 nt



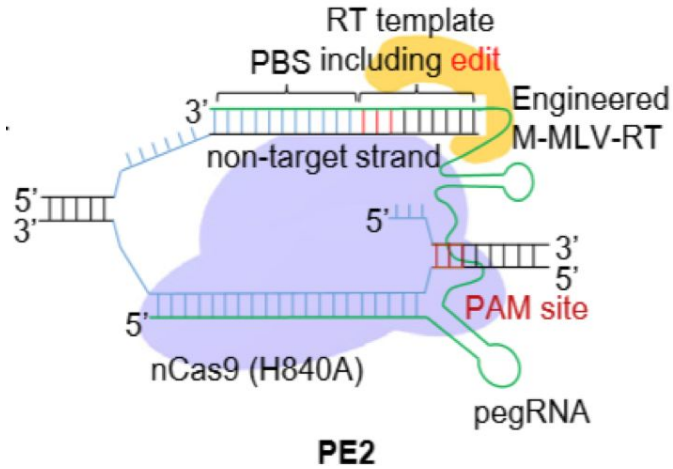
Could introduce:

- Transversion point mutations (Efficiency: 0.7 to 5.5%)
- Insertions and deletions (Efficiency: 4 to 17%)

Second Generation of Prime Editor (PE 2)

Improve prime editing efficiency by engineering the RT

- Replaced original M-MLV RT with an engineered one (6 mutations)



Increased efficiency in:

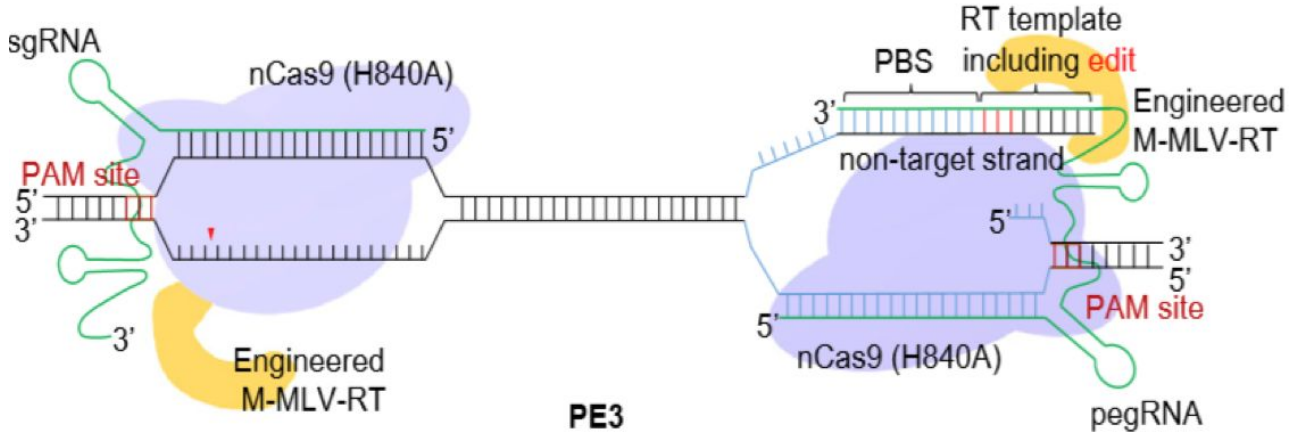
- Point mutations (up to 5.1-fold)
- Insertions, deletions

Third Generation of Prime Editor (PE 3)

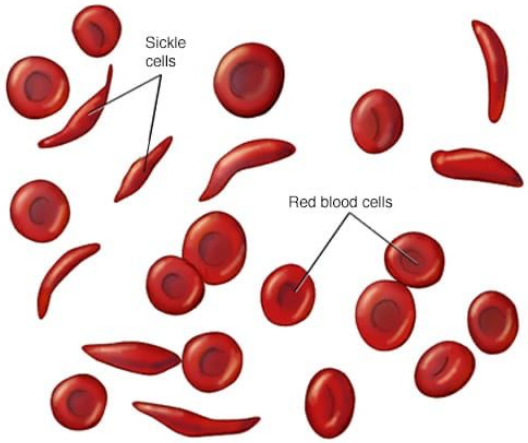
To ensure edit was integrated: use Cas9 nickase to nick the non-edited strand

- Tested a variety of nick locations on non-edited strand
- Optimal: ~50 bp away from the original nick

Increased editing efficiency by 1.5 to 4.2 folds in comparison to PE 2



Sickle Cell Disease



Caused by an A•T-to-T•A transversion mutation in *HBB* gene

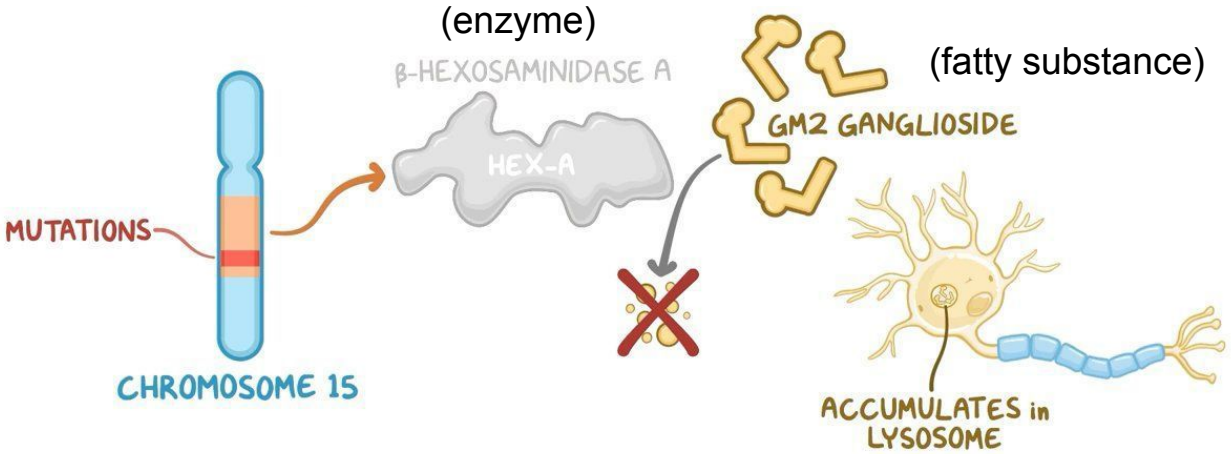
Experiment (Anzalone *et al.* 2019):

1) Used PE3 to insert mutation into HEK293T cells with 44% efficiency and 4.8% indels
2) Treated cell lines homozygous for the mt *HBB* allele with PE3

Able to get editing efficiencies up to 58% with 1.4% indels

Tay-Sachs Disease

4-bp insertion (TATC) in the *HEXA* gene



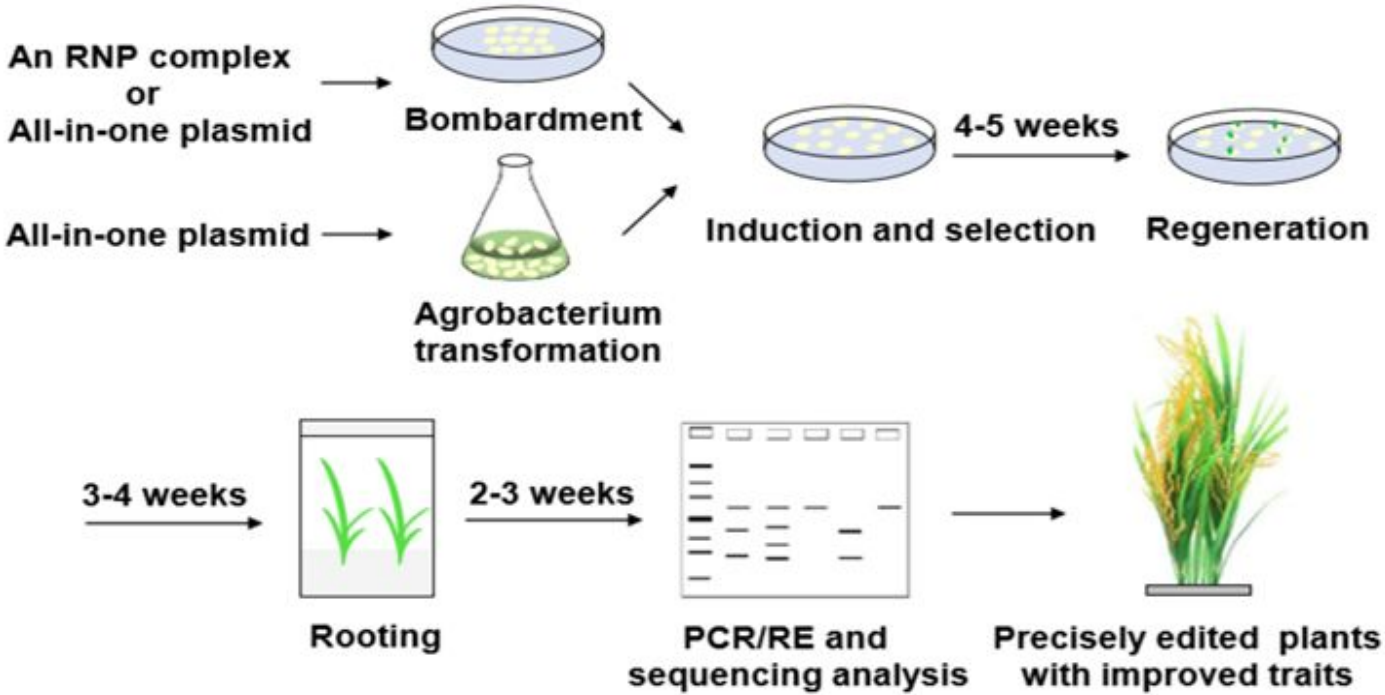
https://www.osmosis.org/learn/Tay-Sachs_disease_(NORD)

Experiment (Anzalone *et al.* 2019):

1) Used PE3 to insert 4-bp with 31% efficiency and 0.8% indels
2) Homozygous cell lines were used to correct insertion with PE 3

Able to get editing efficiencies up to 33% efficiency and 0.32% indels

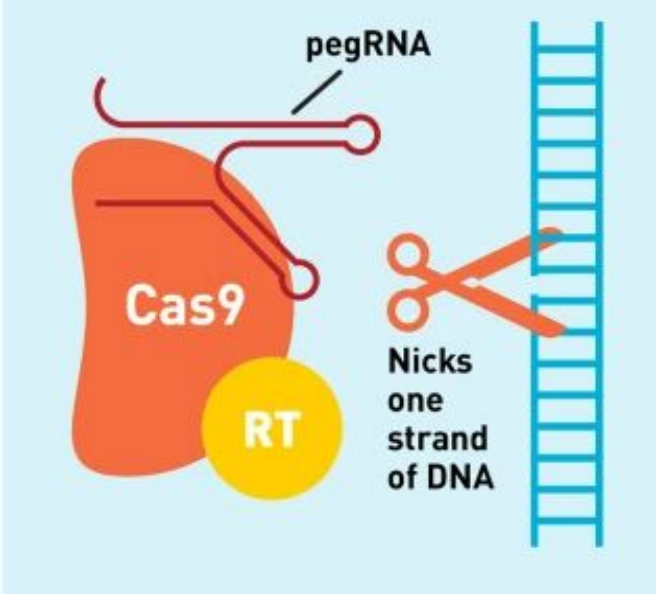
General Steps of Prime Editing in Plants



Potential Applications in Plant Biology Research

Gene correction

Protein engineering



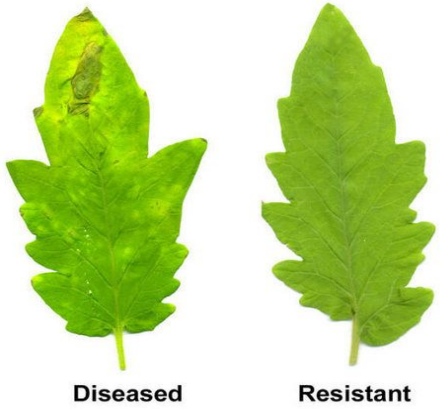
Gene knockout

Cis element engineering

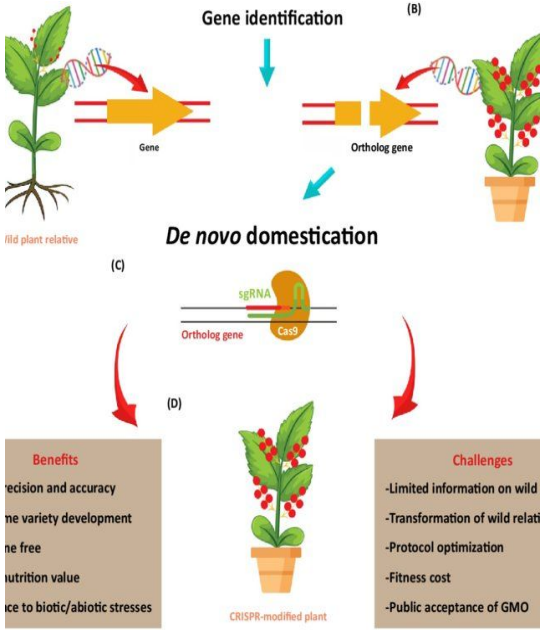
Directed molecular evolution

cont...

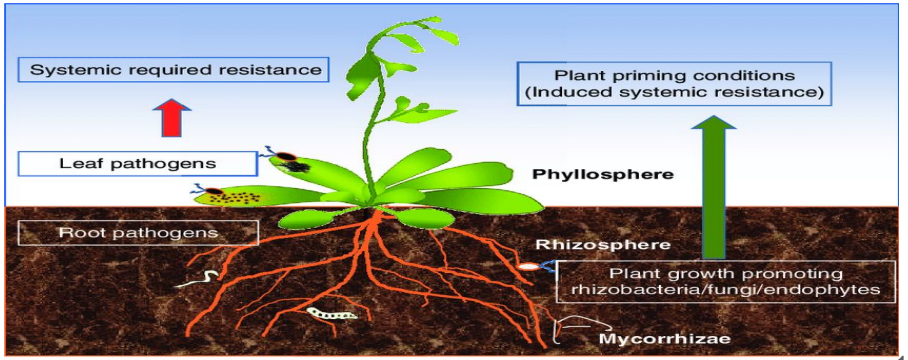
Disease resistance



De novo crop domestication



Plant microbe interaction



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doi: 10.1111/pbi.13399

Case study 1

Brief Communication

Engineering herbicide resistance via prime editing in rice

Haroon Butt, Gundra Sivakrishna Rao, Khalid Sedeek, Rashid Aman, Radwa Kamel and Magdy Mahfouz

Laboratory for Genome Engineering and Synthetic Biology, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

OsALS

Bispyribac sodium

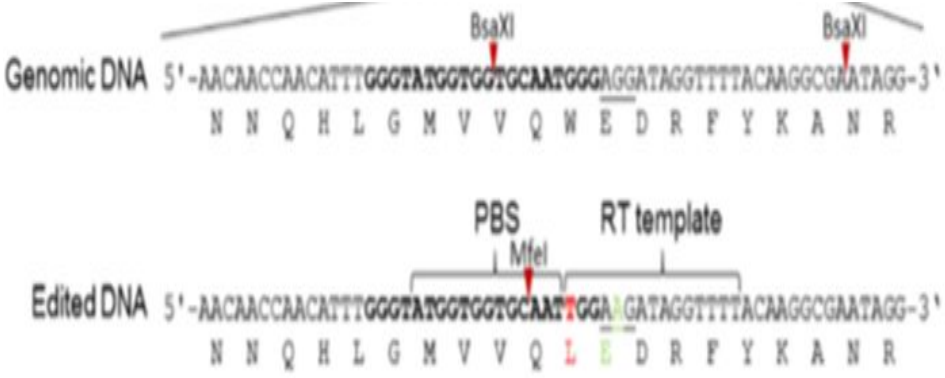
OsIPA

Reduces the number of unproductive tillers and improves rice yield

Engineer herbicide resistance by targeting rice ACETOLACTATE SYNTHASE (OsALS).

👉ALS catalyses the biosynthesis of the branched-chain amino acids

👉 A single amino acid change (W548L)



👉RT template with a length of 15 bp has two substitutions

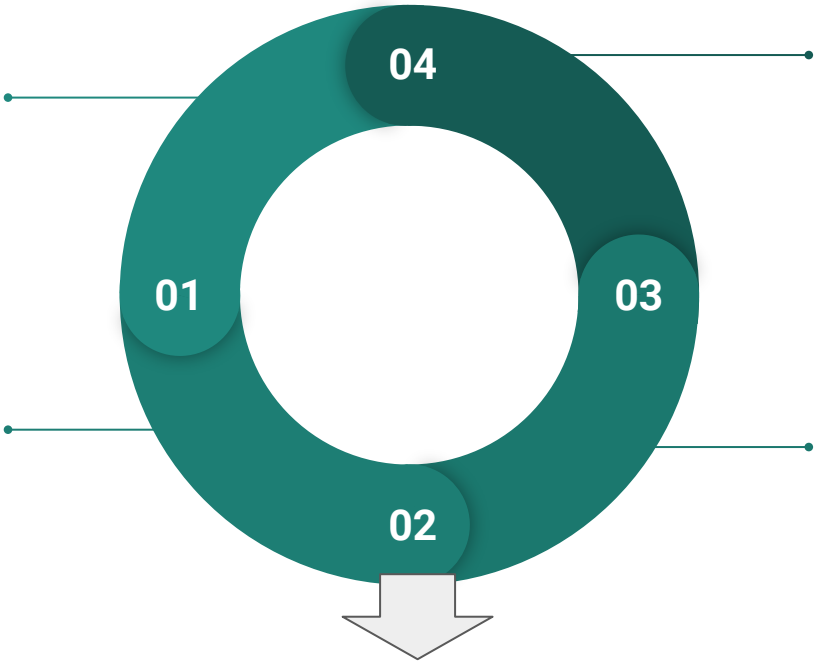
Ø G-to-T (tryptophan 548 to leucine)

Ø G-to-A substitution that destroys the PAM site

Protocol

The PBS length of 13 bp. The pegRNA was expressed in rice vectors under the OsU3 promoter

Cloned the PE2 fragment containing Cas9 (H840A) under the control of the OsUBIQUITIN promoter in rice vector

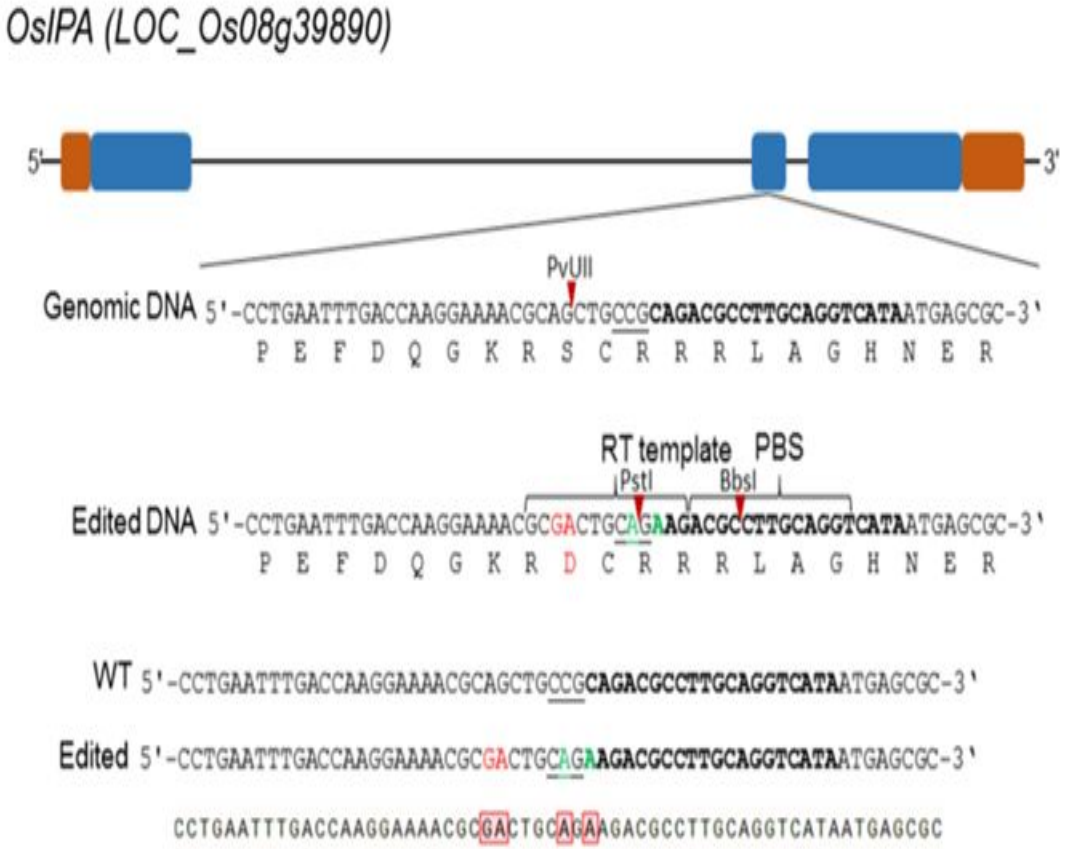


DNA extraction of calli followed by PCR and sequencing

***Agrobacterium* mediated transformation**

Edited OsALS at the target site with an efficiency of 0.26 to 2%

IDEAL PLANT ARCHITECTURE 1 (*OsIPA*) gene



RT length: 20 bp
PBS: 13 bp

Two silent substitutions (CGC to AGA) destroy the PAM site. These mutations destroy a PvuII site and generate Pst1 and BbsI sites

Agrobacterium transformation

Different genes targeted in Rice

Org	Prime editing system	Target genes Editing	frequency	Ref.
Rice	pPE2, pPE3, pPE3b	OsCDC48, OsALS, OsDEP1, OsEPSPS, OsLDMAR, OsGAPDH, OsAAT	≤21.8%	Lin Q (2020)
Rice	pPE2, pPE3, pPE3b	OsPDS, OsACC1, OsWx	0-31.3%	Xu R (2020)
Rice	PE-P1, PE-P2	OsALS, OsACC, OsDEP1	≤26%	Xu W(2020)

Plant Biotechnology Journal

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Case study 2

Brief Communication

Precise genome modification in tomato using an improved prime editing system

Yuming Lu[1,*,†], Yifu Tian[1,†], Rundong Shen[1], Qi Yao[1], Dating Zhong[1], Xuening Zhang[1] and Jian-Kang Zhu[1,2,*]

[1]Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai, China

[2]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA

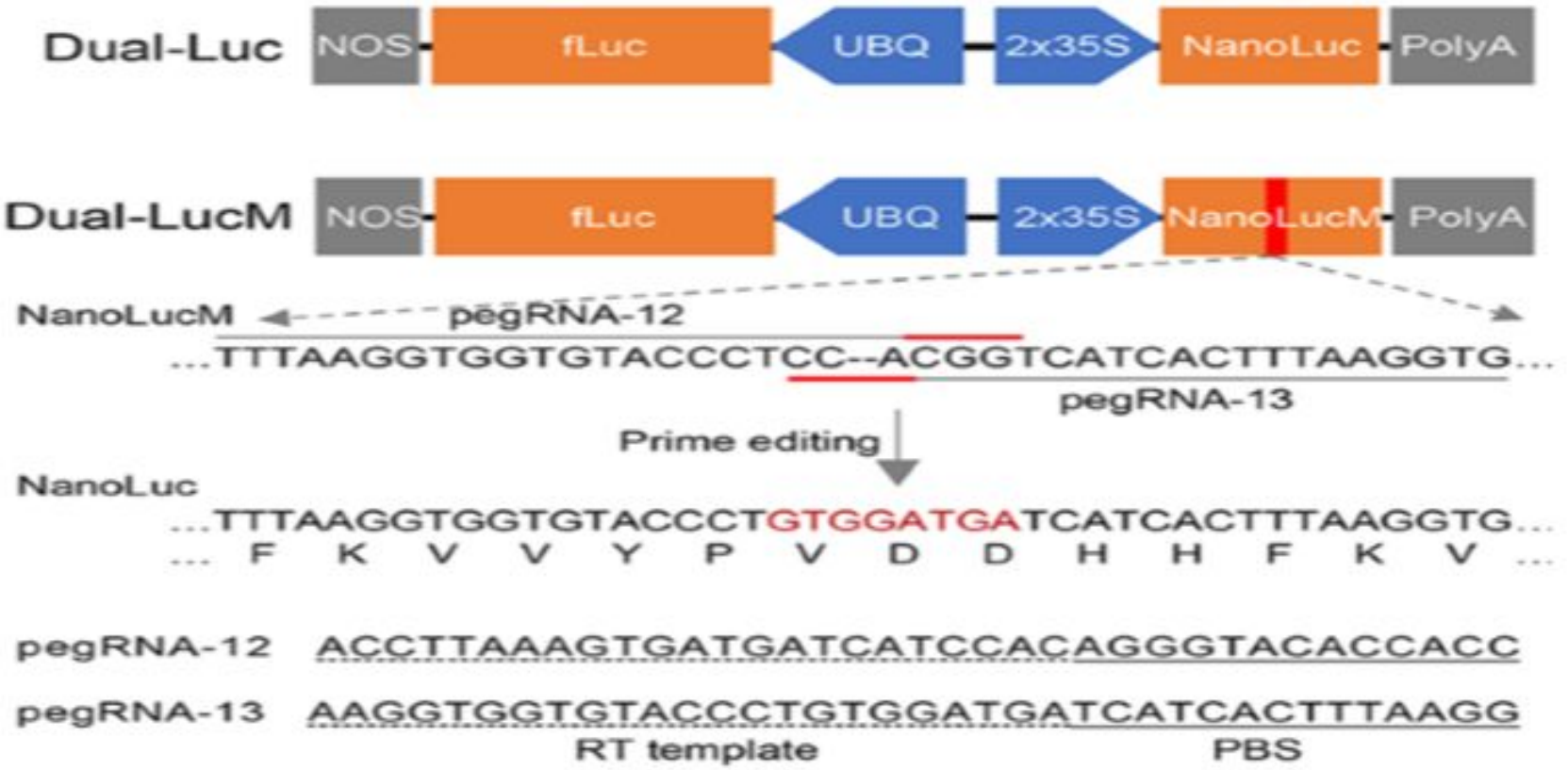
GAI

ALS2

PDS2

6.7% and 3.4% efficiency of editing

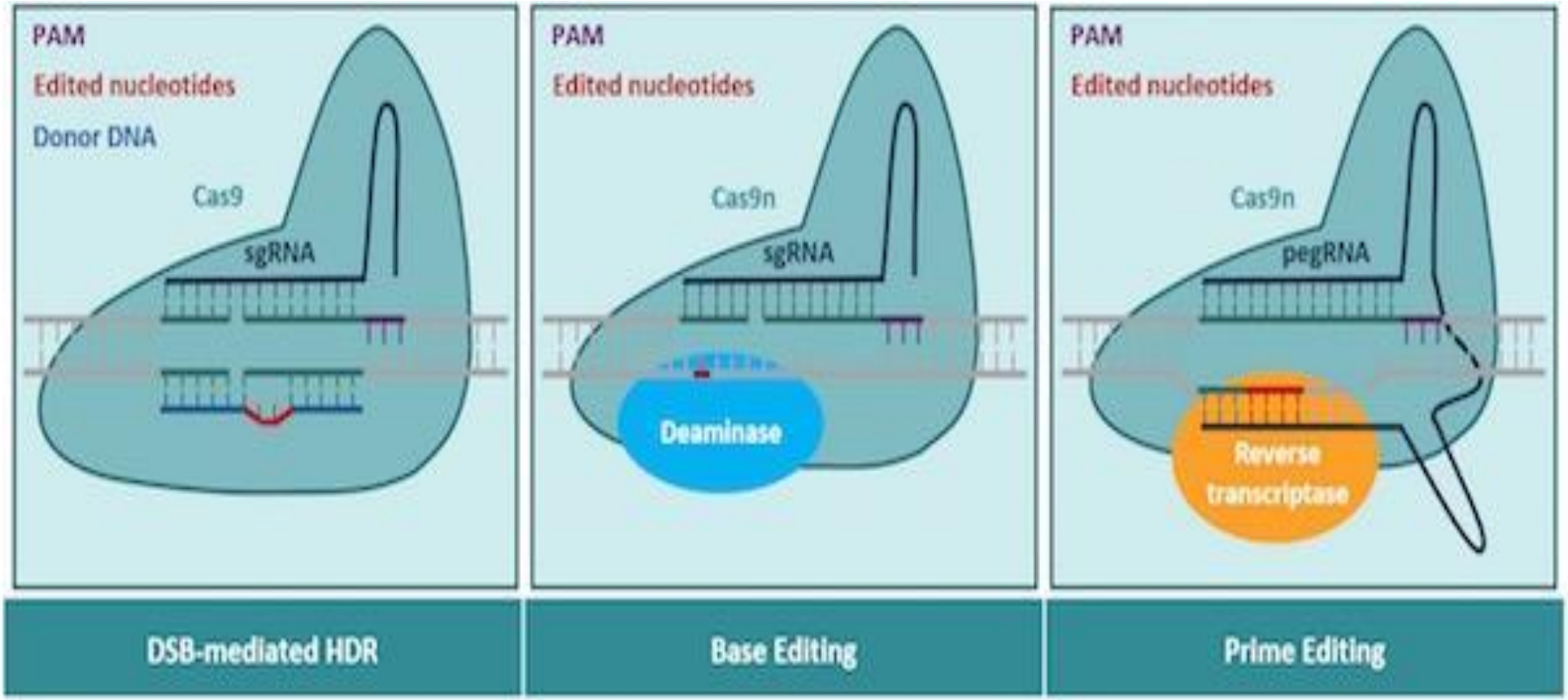
Dual-luciferase reporter system was constructed



Prime Editing vs. CRISPR-Cas9

Prime editing	CRISPR-Cas9
Nicks only one of the two strands of DNA	Cuts both strands at target site
Targeting apparatus is pegRNA	Targeting apparatus is gRNA
Employs DNA mismatch repair	Relies on non-homologus end joining (NHEJ) or homology dependent repair (HDR)
DNA repair is Error free	NHEJ is Error prone
Length of edits: <100bp	Length of edits: 300-1000bp

Comparison of PE with other Gene Editing Technology



Progress of Genome Editing

Meganucleases : **1990s**

Oligonucleotide directed Mutagenesis : **1990s**

Zinc Finger nuclease (ZFNs): mid **1985**

Transcription activator like effector nuclease (TALENs) **2010s**

Clustered regularly spaced short palendromic repeats (CRISPER) **2013**

CBE was discovered for the first base editing tool in human cell for C to T conversion **2016**

Base editors **2016**

•ABE was developedfor conversion of A to G
•Successful conversion of CBE in plants
2017

Sucessful application of ABE in plants
2018

prime editors in plants
2020

Conclusion

- Can insert DNA sequences with single-nucleotide precision without DSBs
- In principle, can correct about 89% of pathogenic human genetic variants
- Can be used to engineer specific and desired traits in crops
- Precise, lower chance of unwanted edits, easy to use and implement

Prime editing is new tool that can be used to expand the scope of genome editing.