Advances in CRISPR/Cas system for targeted genetic modifications: PRIME EDITING

ANSC-691 Seminar

Presenters:

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Introduction

Progress in Genome Editing

Meganucleases: 1990s

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Zinc Finger Nucleases (ZFNs): mid-1985

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Transcription activator like effector nucleases (TALENs): 2010s

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Clustered regularly interspaced short palindromic repeats (CRISPR): 2013

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Base Editing: 2016

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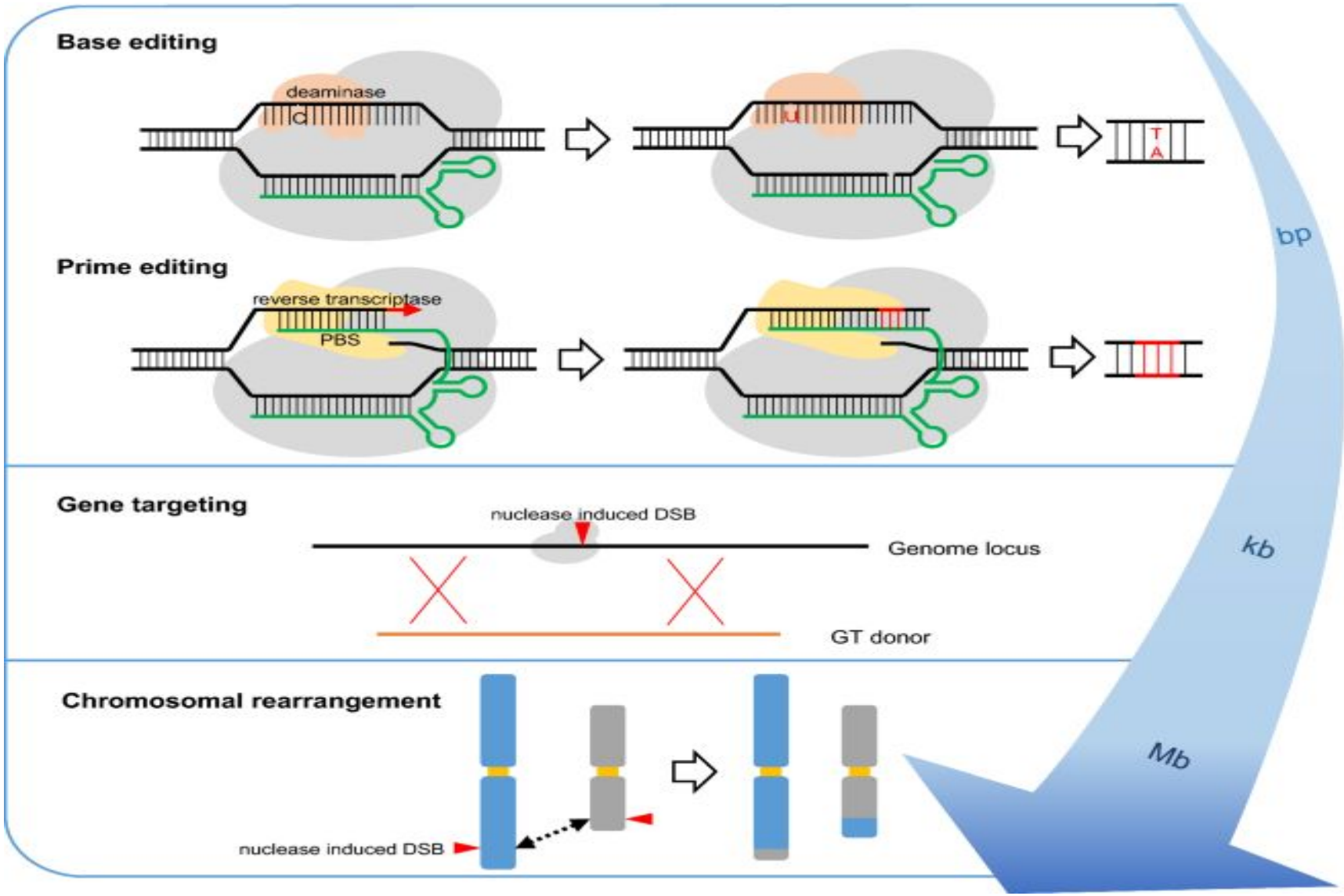
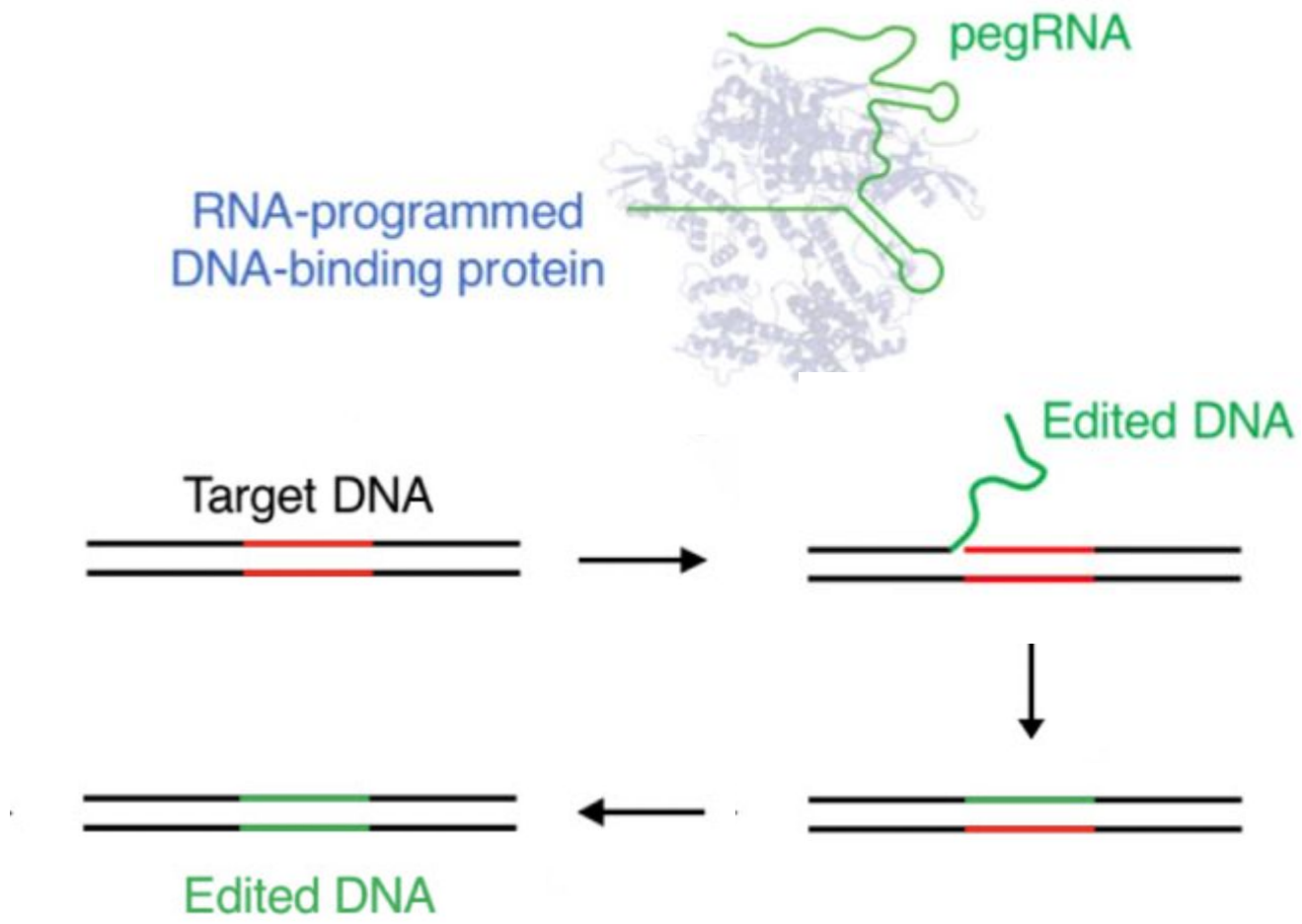
Prime Editing: 2020

Advances in CRISPR



Huang and Puchta, 2021

An overview of prime editing



Excision by endonuclease

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Target site: one edited and one unedited strand

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A different guide RNA directs the prime editor to nick the unedited strand

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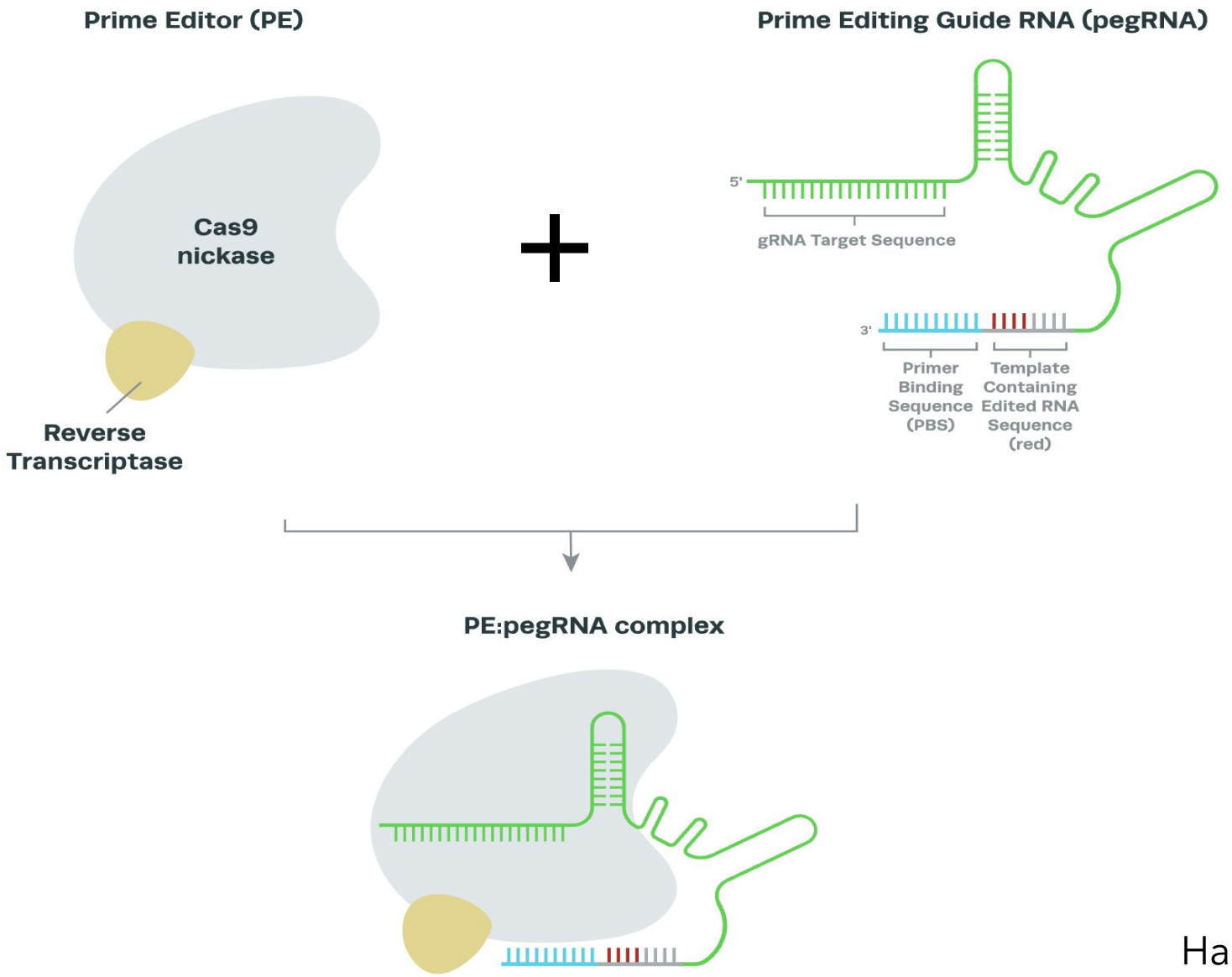
The edited strand is used as template to remake the nicked strand

Tsang, 2019

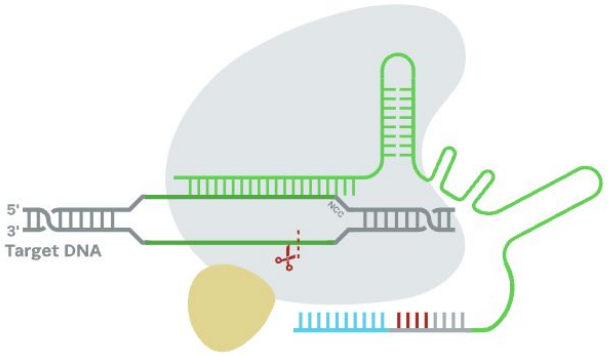
Components of prime editing



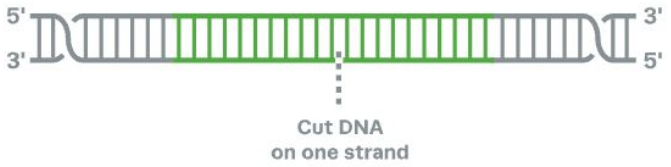
Hassan et al., 2020

Steps in prime editing

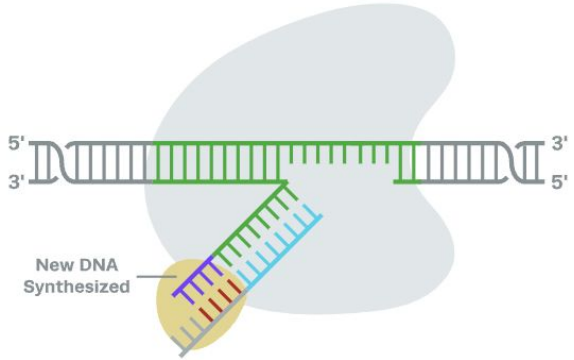
1. PE:pegRNA Complex Binds to Target DNA



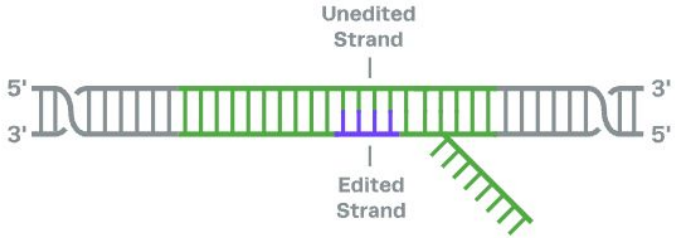
2. Cas9 Nickase Cuts One Strand of DNA



3. Reverse Transcription of RNA Incorporates Desired Sequence into Target DNA



4. Edited Strand is Incorporated. Original DNA is Cleaved by Cellular Endonuclease

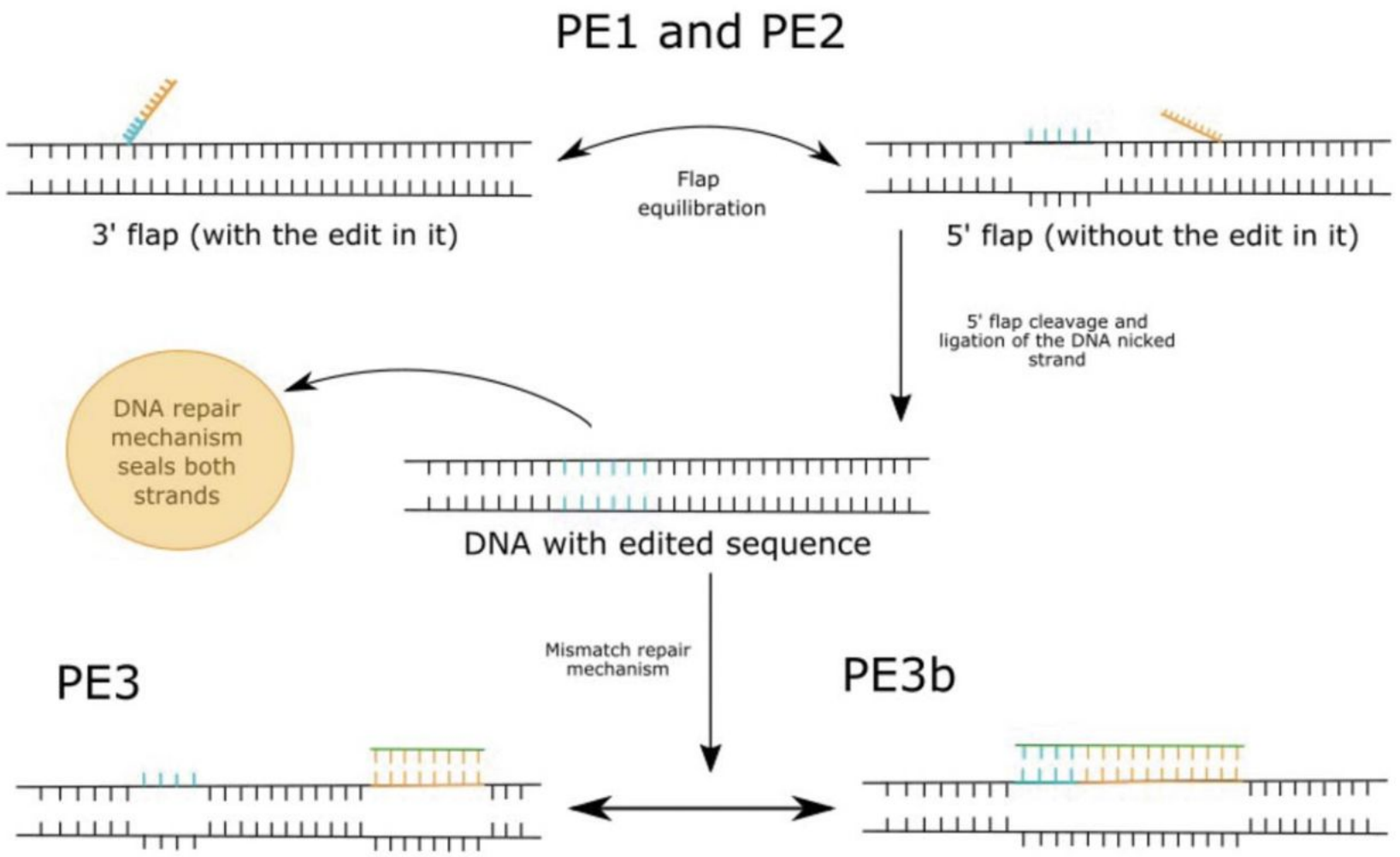


5. Unedited Strand is Repaired to Match Newly Edited Sequence*

*using PE3, PE3b systems

Ochoa-Sanchez et al., 2021

Prime editing generations



Ochoa-Sanchez et al., 2021

Applications

Advantages of prime editing

Less constrained by PAM sequence location

More versatile and precise than base editing

Fewer byproducts

More efficient than HDR

Tsang, 2019

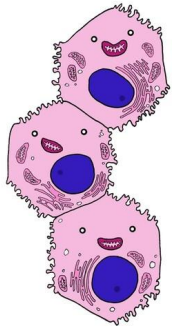
Applications in medicine

Therapeutics

Duchenne muscular dystrophy

Sickle cell disease

Tay-Sachs disease



Cancer research

Organoid production

Introduce desired mutations in hepatocytes and colon tumor organoids

Tested in human adult stem cells and cancer cell lines

Genome editing for parasitic worms

Approaches for targeted mutations

Site-specific integration of trans-genes

High-throughput screening for gene functional analysis

Ochoa-Sanchez et al., 2021

Molecular Therapy
Nucleic Acids
Original Article

Modeling a cataract disorder in mice with prime editing

Jianxiang Lin,[1,7] Xingchen Liu,[2,3,7] Zongyang Lu,[2,7] Shisheng Huang,[4] Susu Wu,[1] Wenxia Yu,[4] Yao Liu,[5] Xiaoguo Zheng,[6] Xingxu Huang,[4] Qiang Sun,[2] Yunbo Qiao,[1] and Zhen Liu[2]

Microinjection, embryo in-vitro culturing, and embryo transfer

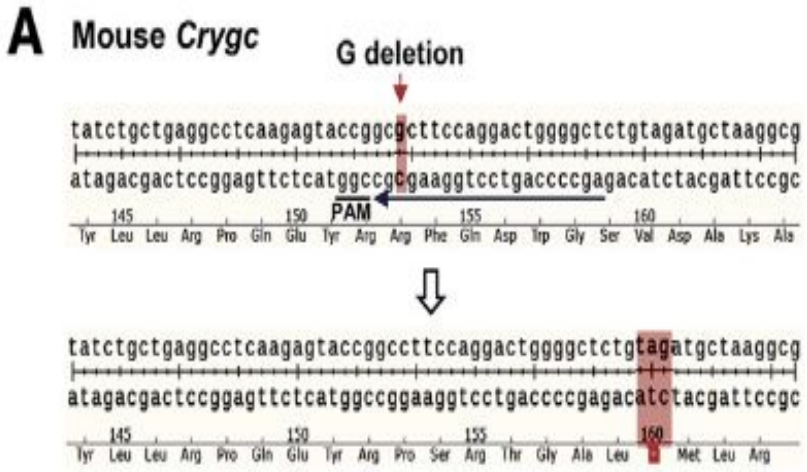
Cell culture and transfection

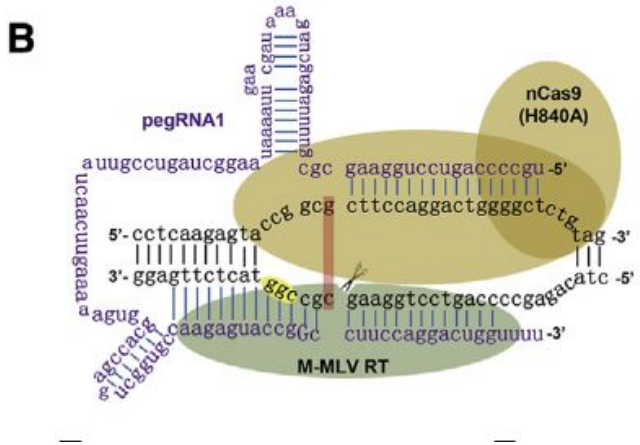
Genomic DNA extraction and genotyping

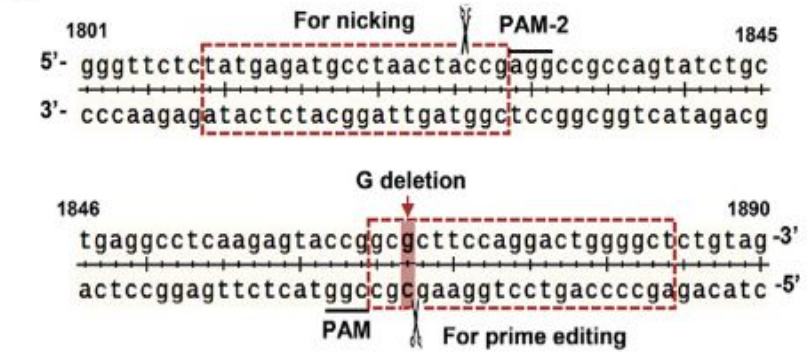
Targeted deep sequencing and elimination of off target activity

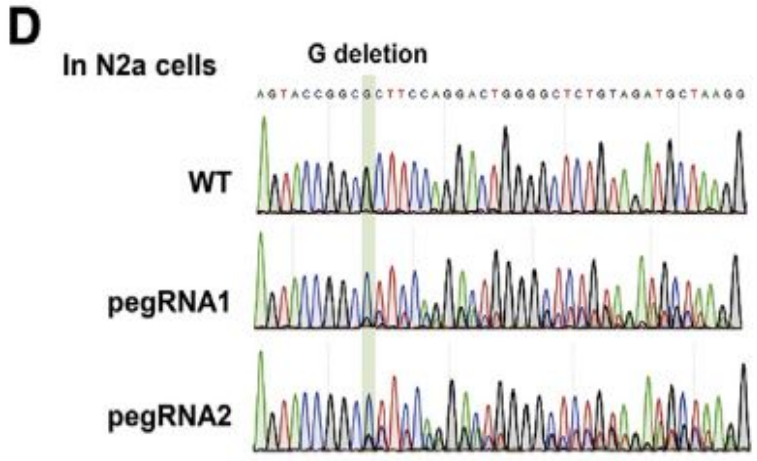
Lin et al., 2021

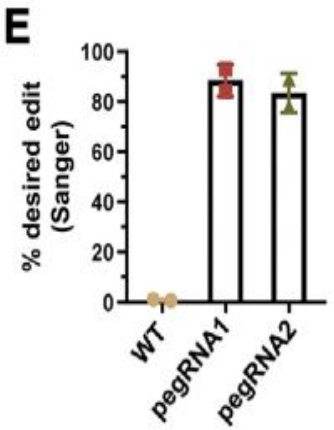
Highly efficient installation of a G-deletion(G-del) mutation

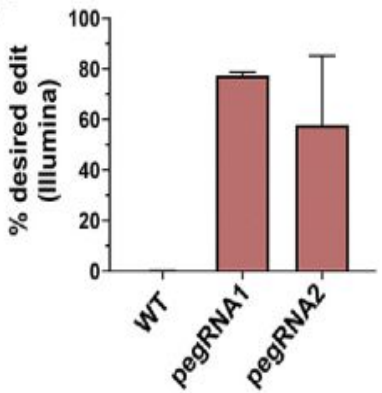












Lin et al., 2021

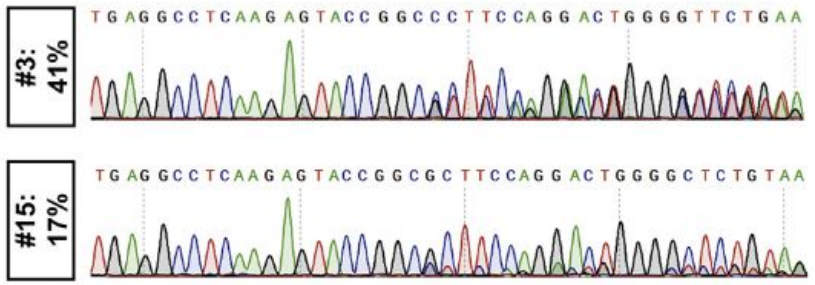
PE3-mediated efficient base deletion to model a cataract disorder in the mouse

A PE2+pegRNA1+nicking sgRNA plasmids

Group	Blastocyst /Injected zygote	Edited/ Sequenced	Editing efficiency (%)
Experiment 1	8/10	2/8	13.8%;100%
Experiment 2	12/13	1/9	22.60%
Experiment 3	13/17	1/13	15.50%

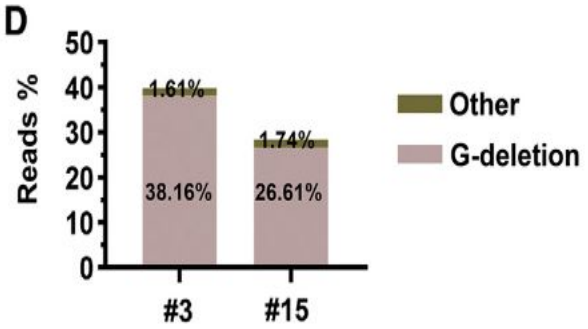
B

Injected zygote	Transferred Embryos	Edited/ Sequenced	Editing efficiency (%)
85	80	2/19	41%;17%



C

D



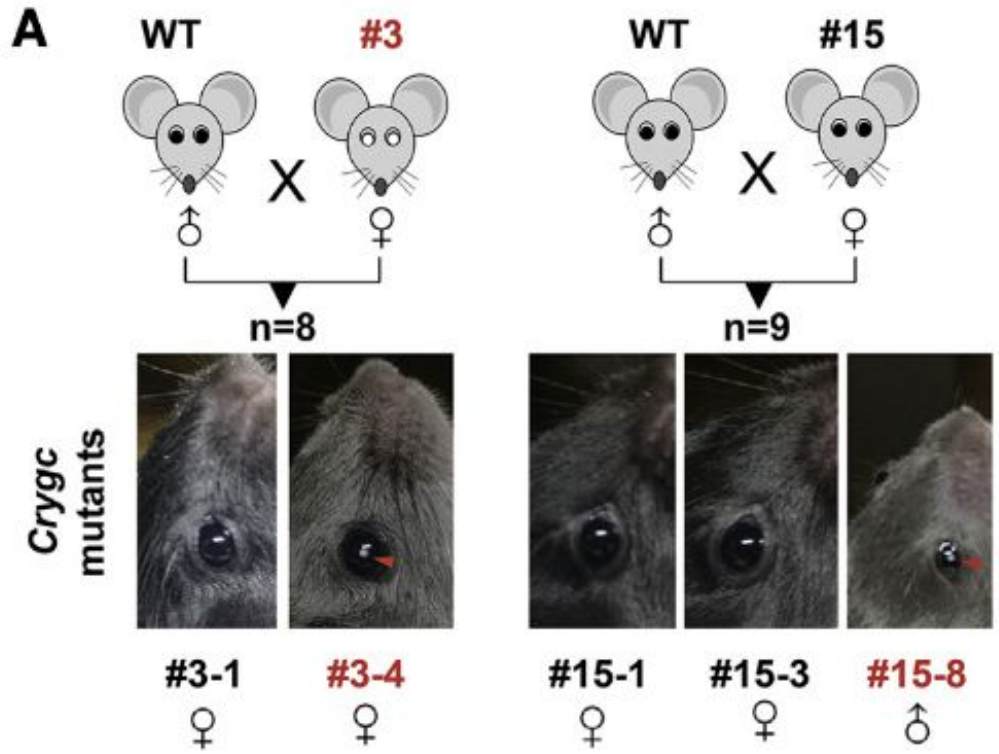
E

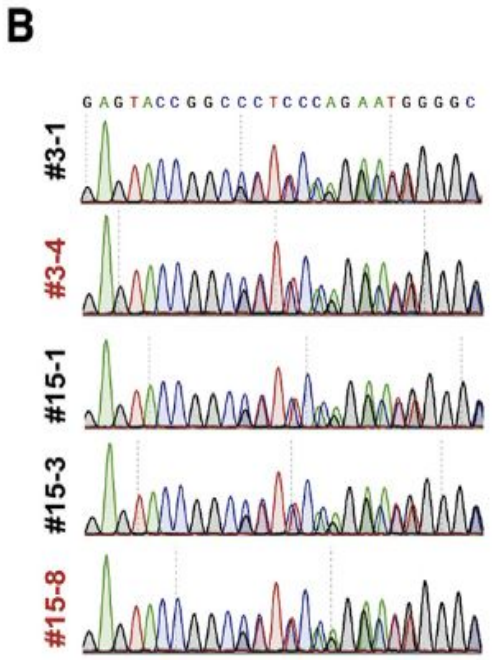
Whole Genome Sequencing	#3	#15
Sequencing coverage	21.1x	18.63x
OT-indel (pegRNA)	0/1413	0/1413
OT-indel (nicking sgRNA)	0/537	0/537

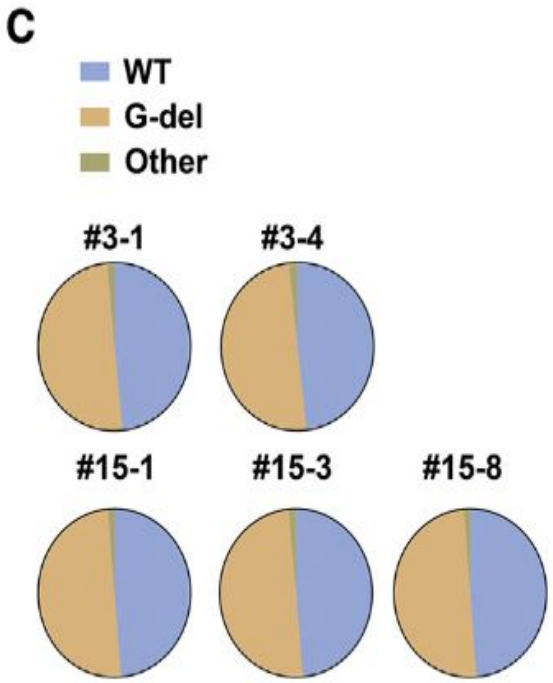
Cas-OFFinder3 (NGG PAM; mismatch ≤ 5)
OT: off-target

Lin et al., 2021

PE3-induced base deletion is transmitted to the next generation







Lin et al., 2021

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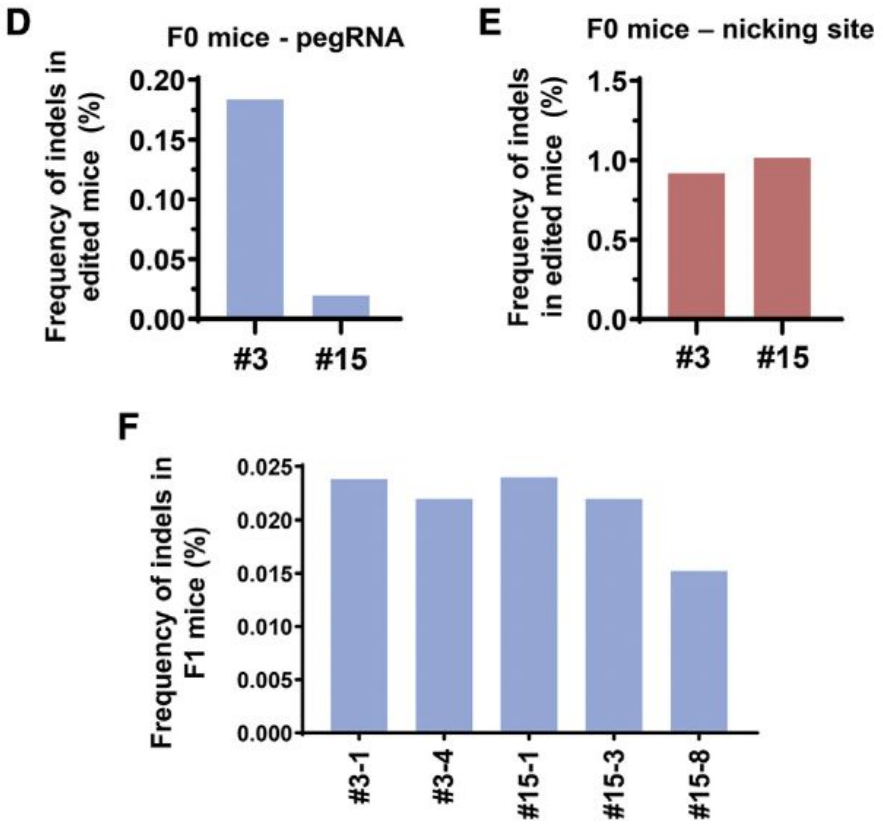
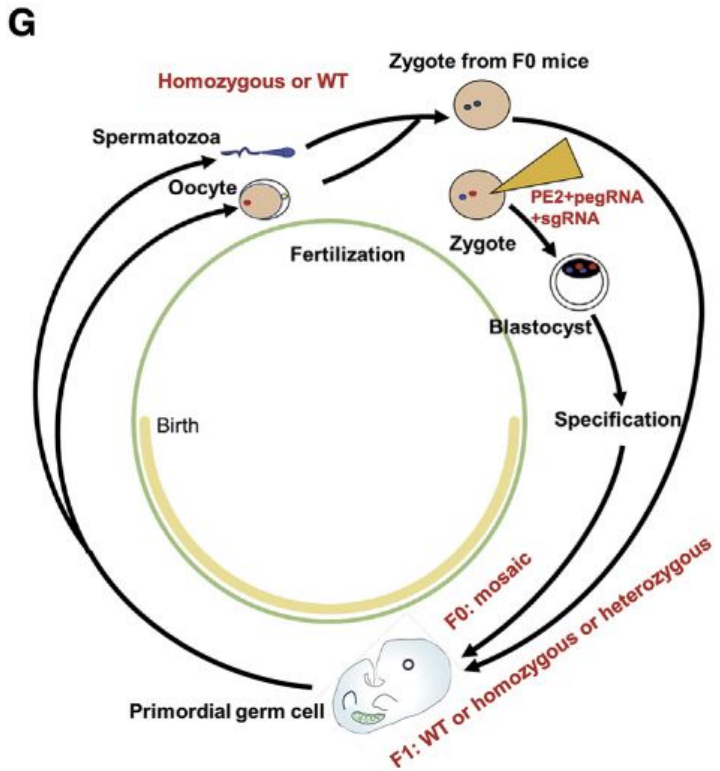
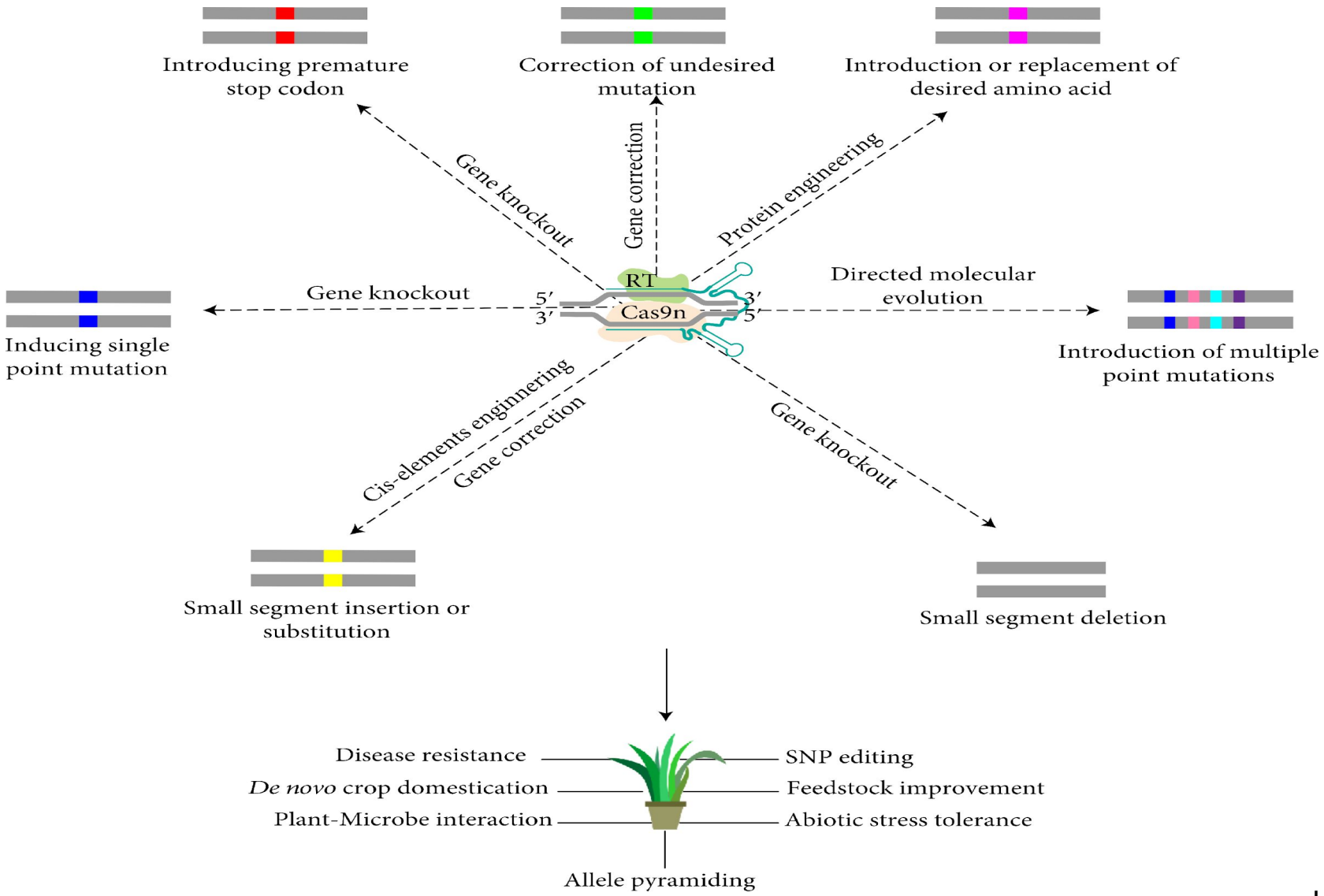




Lin et al., 2021

Applications in plant biology



Hassan et al., 2020

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2021) **19**, pp. 415–417 doi: 10.1111/pbi.13497

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,*]

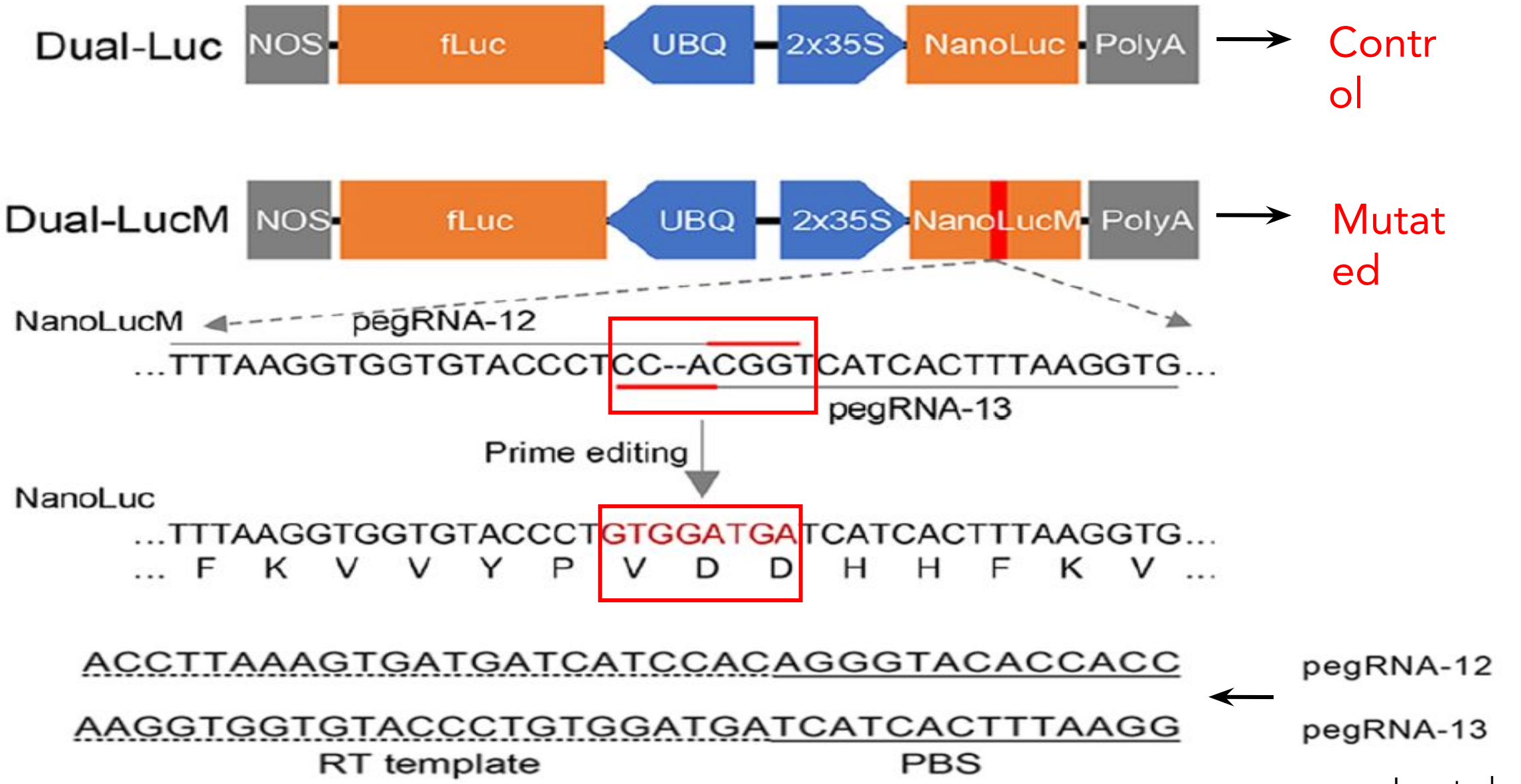
[1]*Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai, China*
[2]*Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA*

GAI *ALS2* *PDS2*

↓ ↓

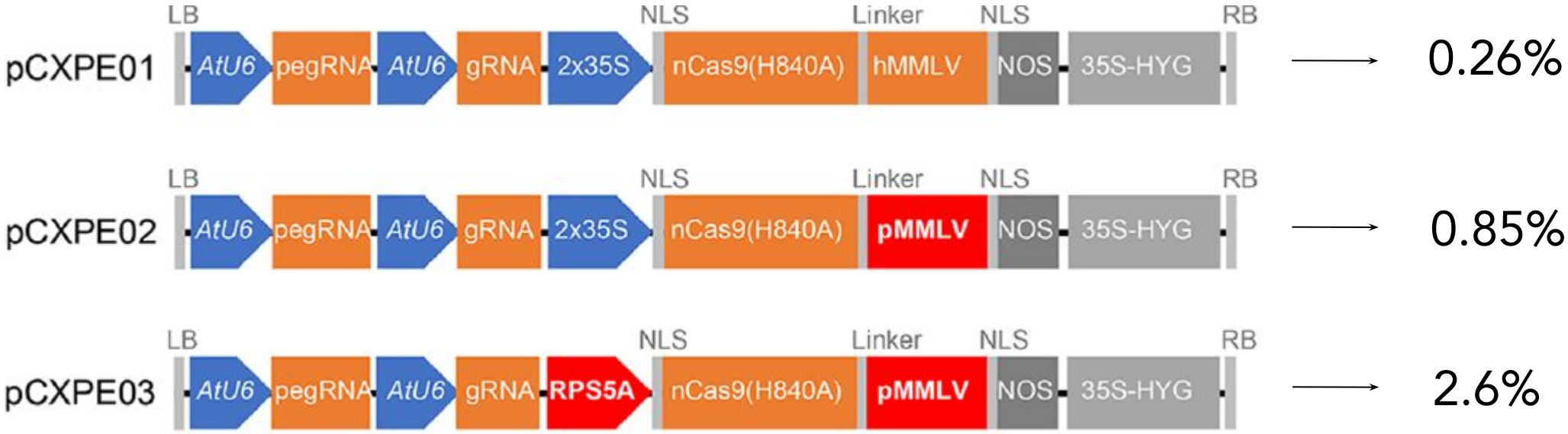
6.7% and 3.4% efficiency of editing

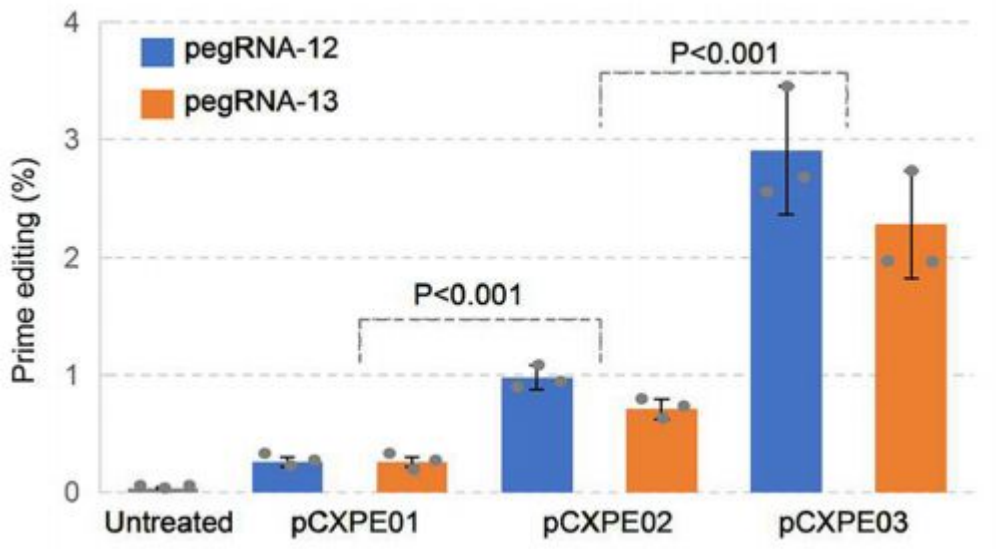
Proof of concept



Lu et al., 2021

Optimization for improved editing efficiency





Lu et al., 2021

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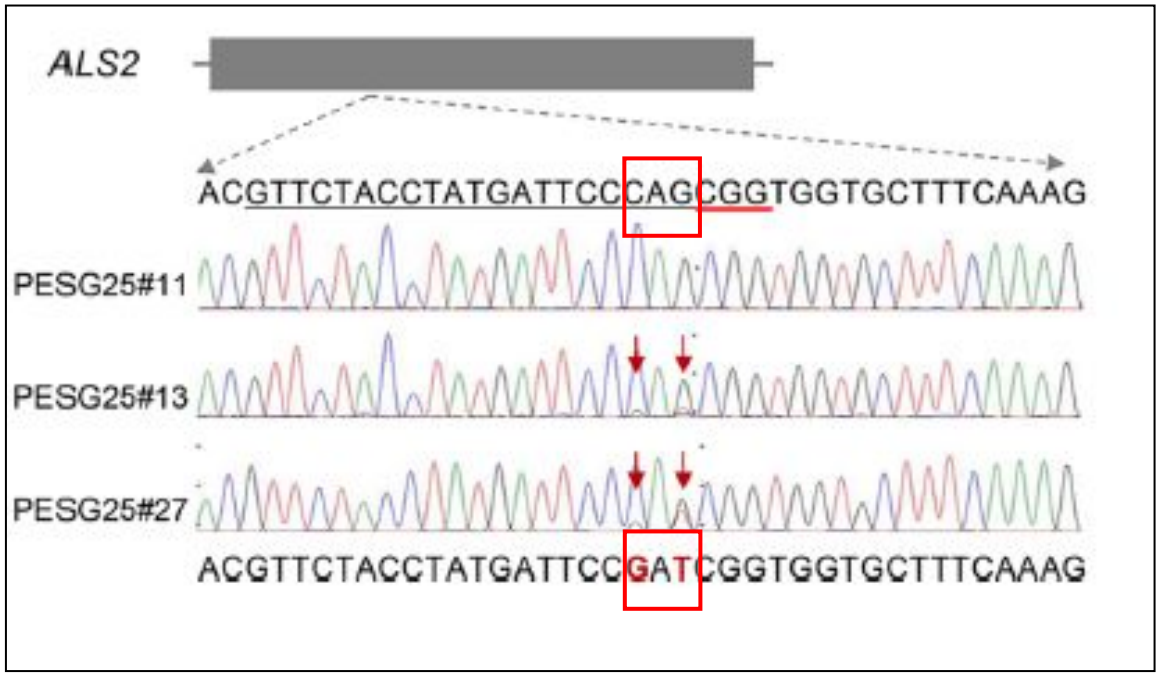

Lu et al., 2021

Conclusion

Comparison with other technologies

	DSB-mediated HDR	Base Editing	Prime Editing
Components	•Cas9 •gRNA •Donor DNA	•Base editor (fusion Cas9 + deaminase) •gRNA	•Prime editor (fusion Cas9 + RT) •pegRNA
Possible modifications	•All precise modifications •Large modifications	•Only transition mutations	•All precise modifications
Design constraints	•Efficiency decreases with distance between PAM and mutation •High efficiency: less than 15nt	•Efficient for mutations 15nt from PAM only	•Efficient for mutations 1nt to more than 30nt from PAM
Potential drawbacks	•High indel rate •Genome-wide off targets •On-target rearrangements	•Bystander editing •Genome-wide off-targets	•Potential transcriptomic dysregulation •Genome-wide off-targets?

Rezza and Armezzani, 2019

Summary

- A promising technology aimed at decreasing the common undesirable effects associated with conventional genome editing approaches.
- Currently, progress has been achieved in improving the efficiency of genome editing by the PE.
- However, PE also introduces new challenges such as unwanted mutations and the limitation of large DNA insertions that conventional CRISPR-Cas9 is capable of.
- Therefore, further research is required to optimize PE tools and maximize its efficiency.

THANK YOU