中国烟草
CHINA TOBACCO

云南省烟草农业科学研究院
YUNNAN ACADEMY OF TOBACCO AGRICULTURAL SCIENCE



CORESTA CONGRESS 2022

# *Application of protoplast technology facilitates the CRISPR-Cas9 mediated gene replacement in Nicotiana tabacum*

Cheng Yuan

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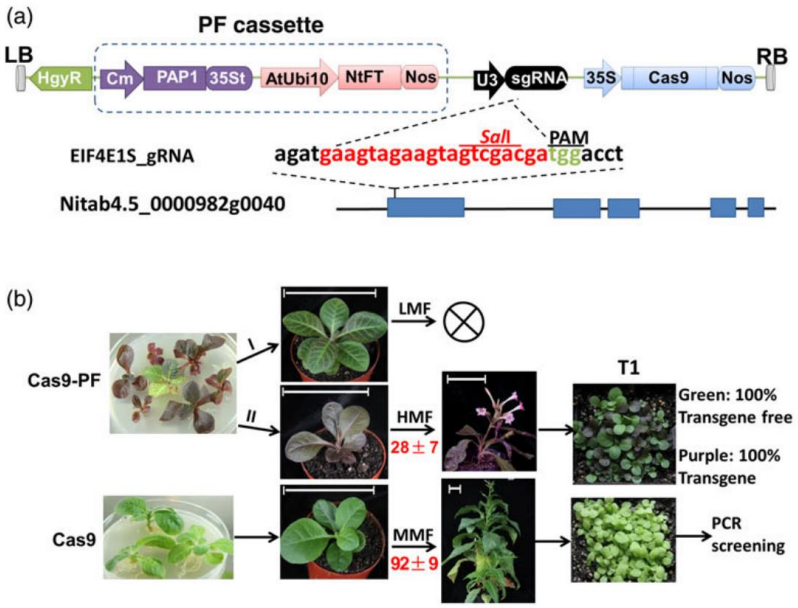
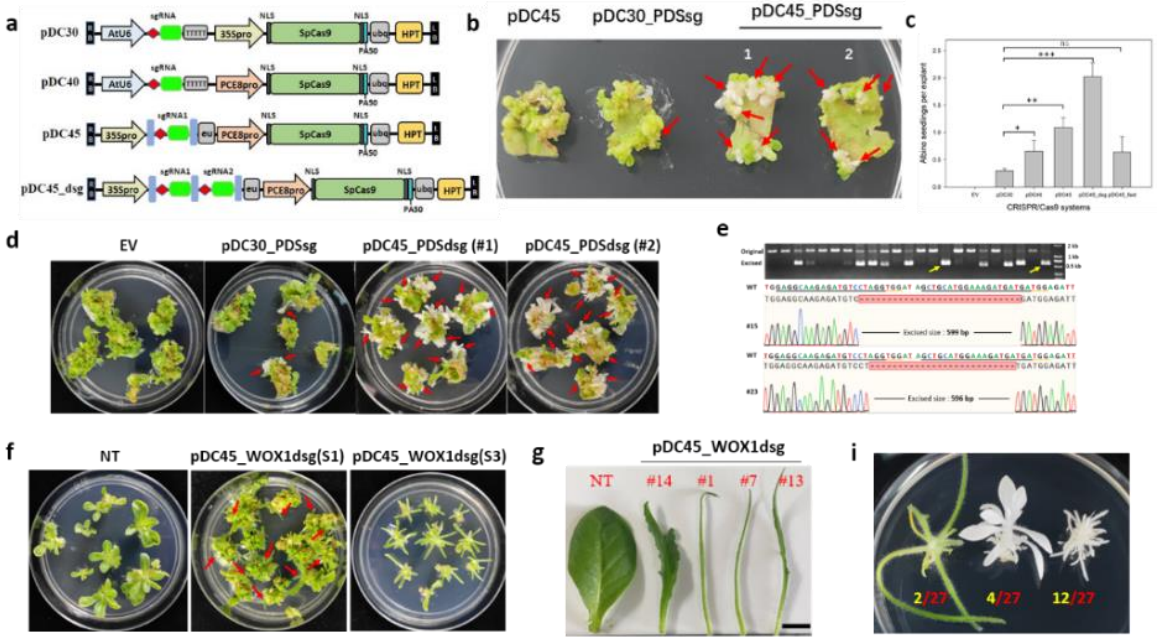
In the city of Kunming

# CRISPR-Cas9 application in tobacco




Liu et al., 2019



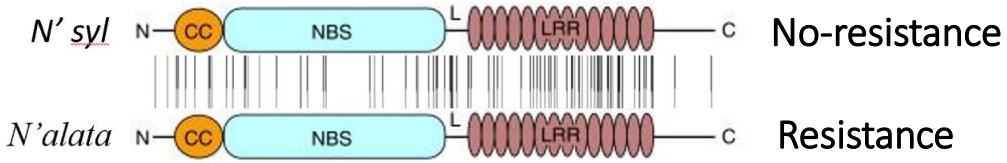

Li et al., 2022

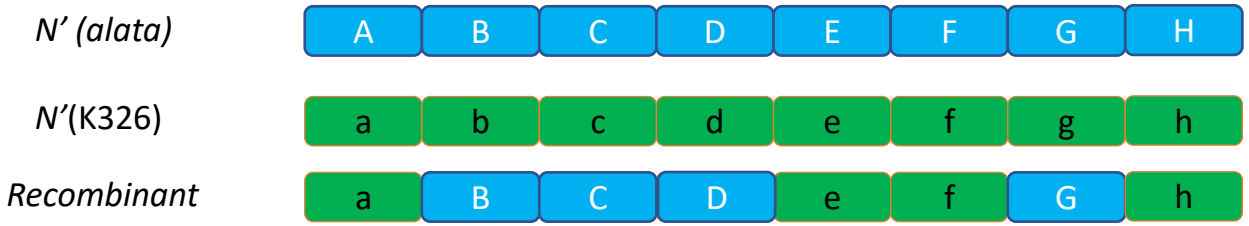
CRISPR-Cas9 has been widely applied in breeding, mainly gene knock-out, genome editing in tobacco needs new breakthrough.

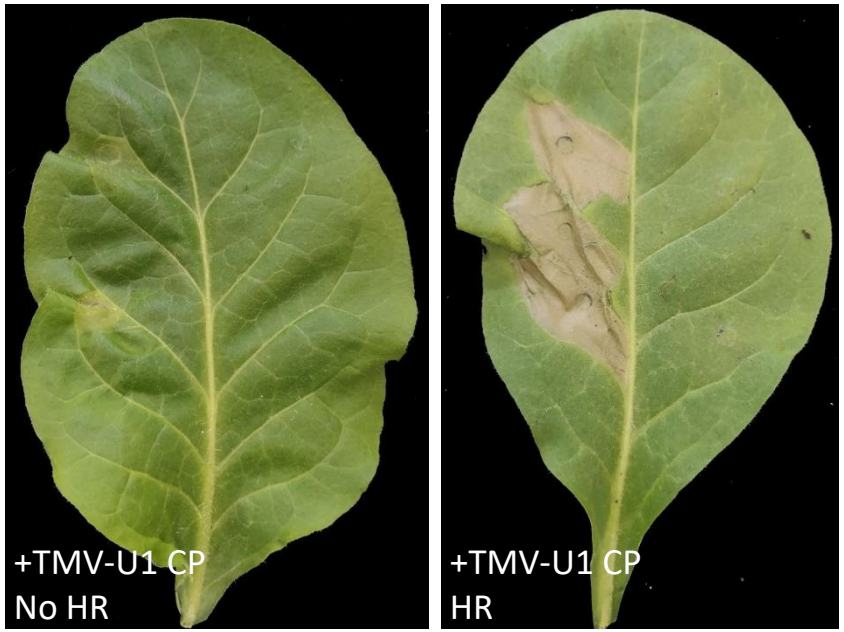
# Background: *N'alata* and *N'* gene



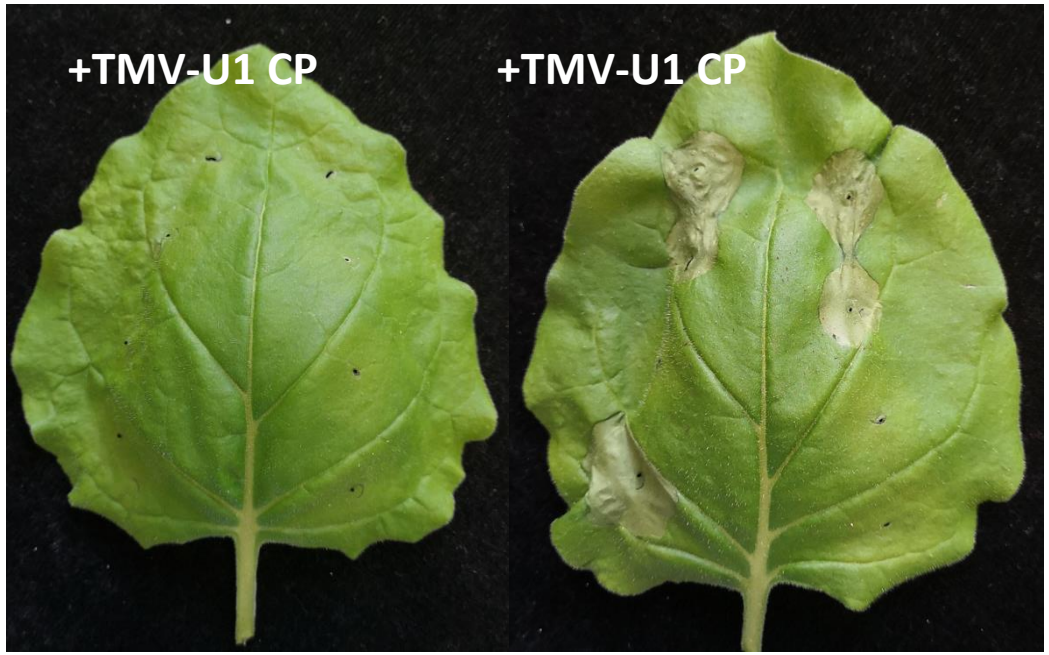
**TMV-U1 Strain: dominant pathogen**
**TMV-Cg Strain**




**K326 ::N'** **K326::N'alata**




**K326 ::N'** **K326:: Recombinant**

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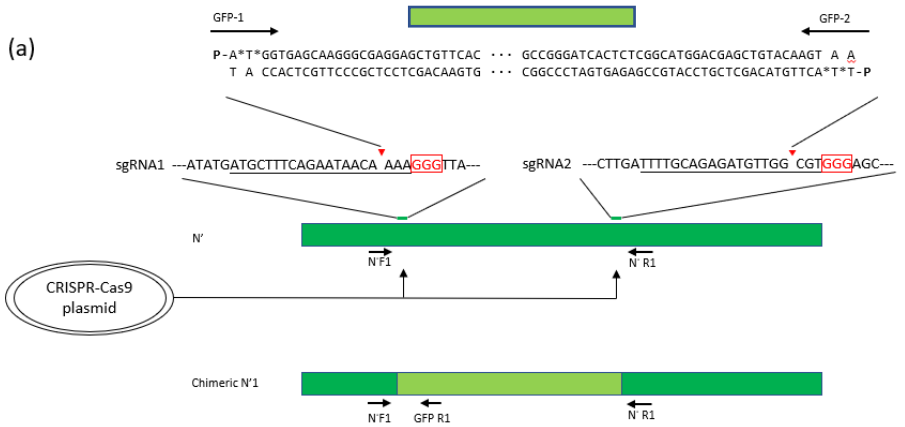
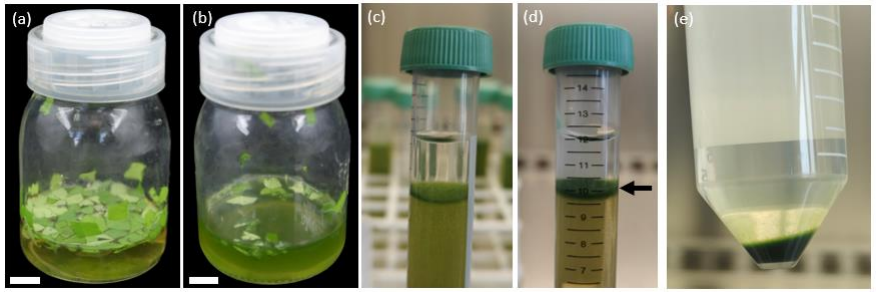
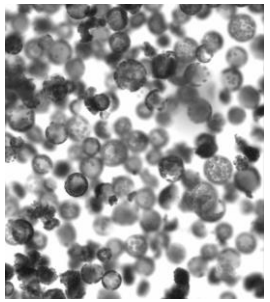
# Establishment of gene knock-in in tobacco

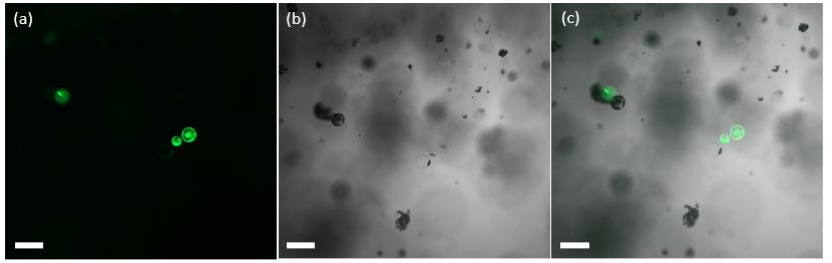
# Establishment of gene knock-in system in protoplast

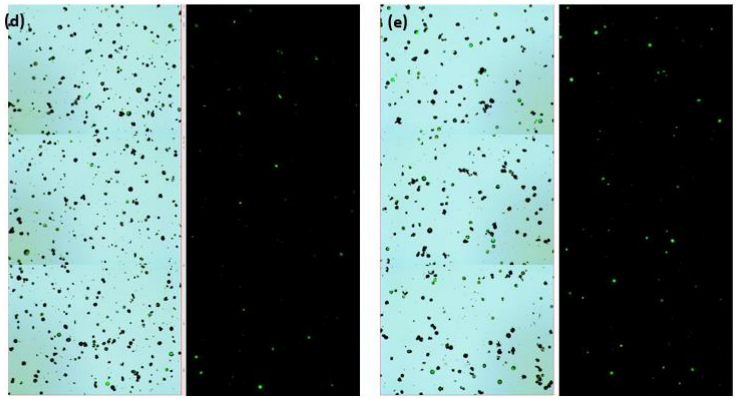



5′-phosphorylated, phosphorothioate-linkages at both 5′



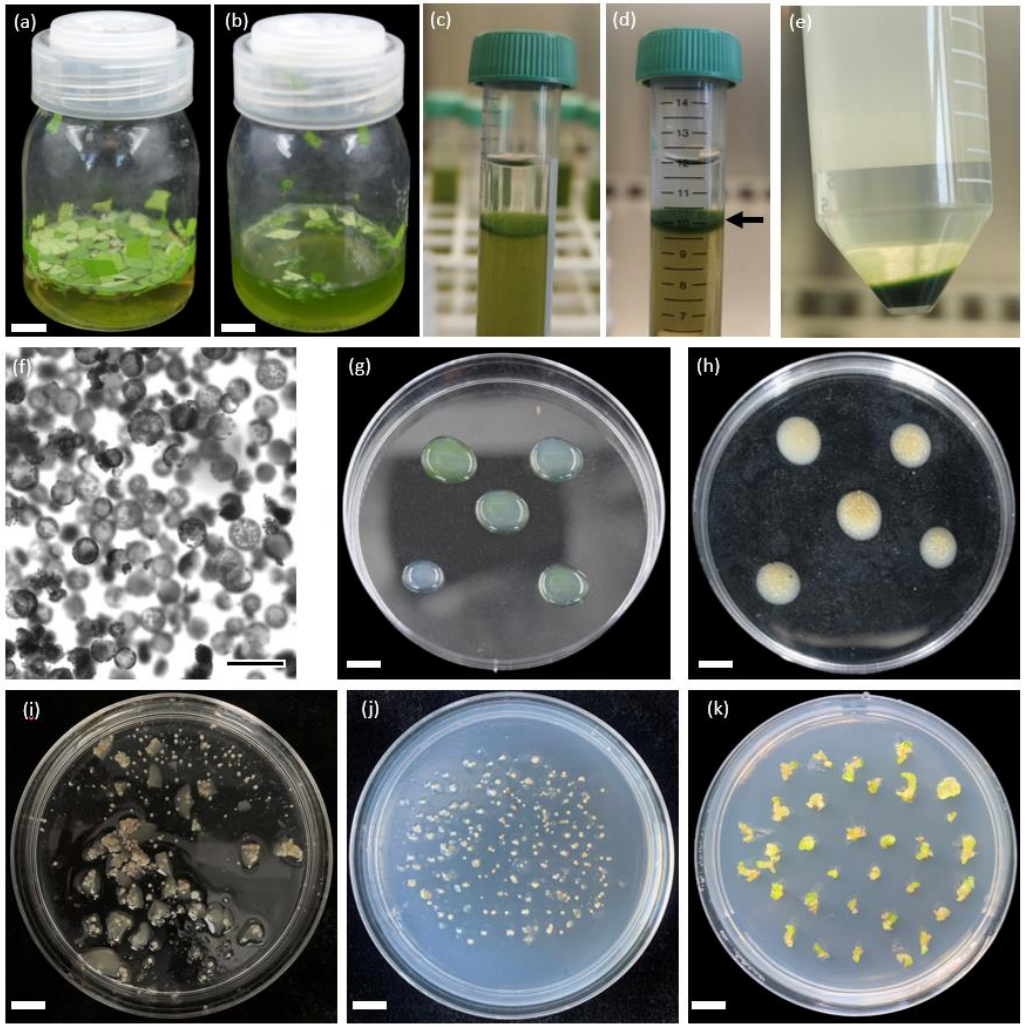


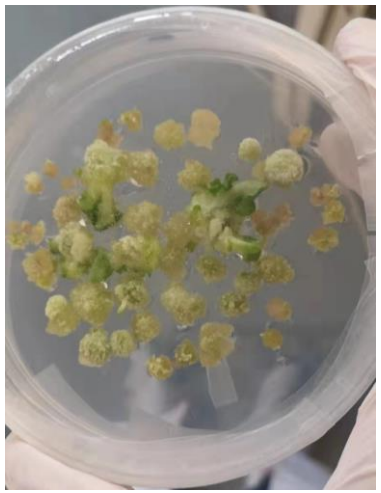




GFP: 1.5% of total cells

GFP: 3% of total cells

# Protoplast transfection and regeneration optimization



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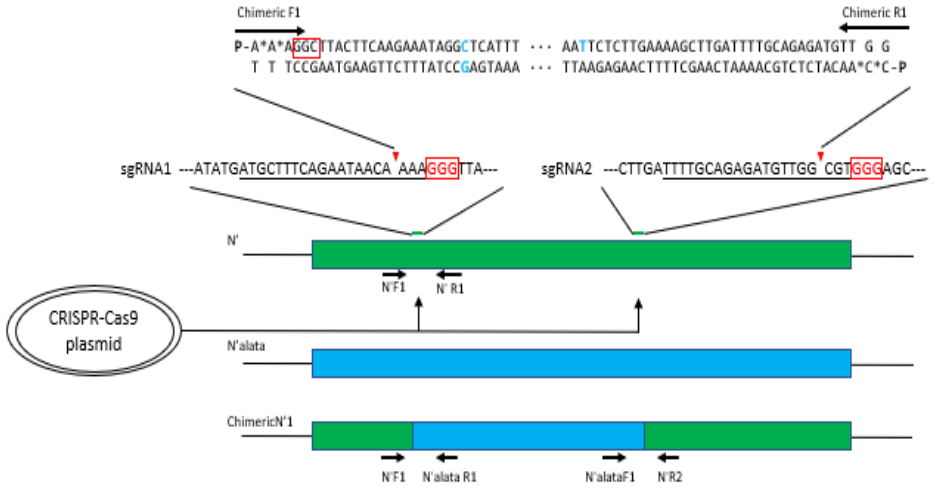
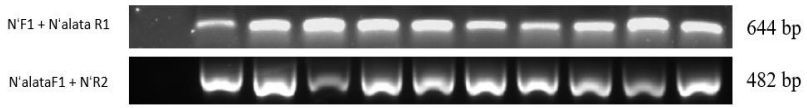
# Application of gene knock-in and plants regeneration

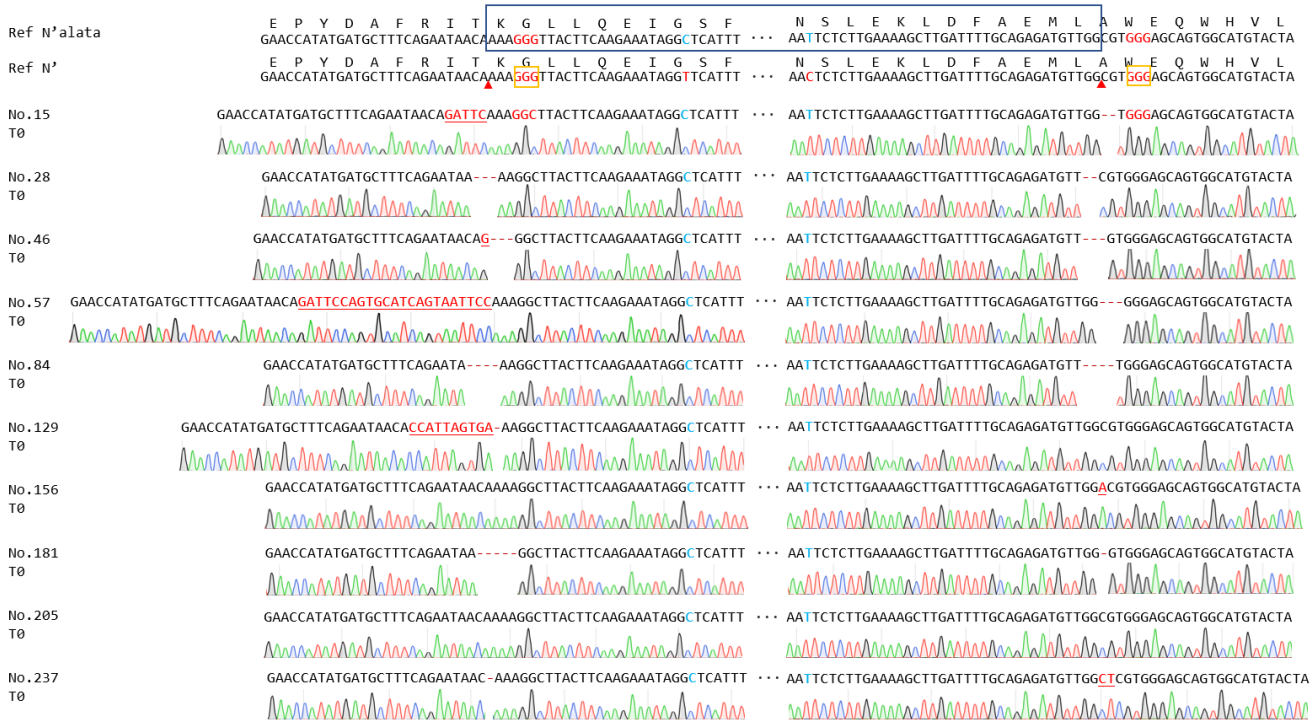
# Gene replacement of *N'* with *N'alata* fragment

5′-phosphorylated, phosphorothioate-linkages at both 5′



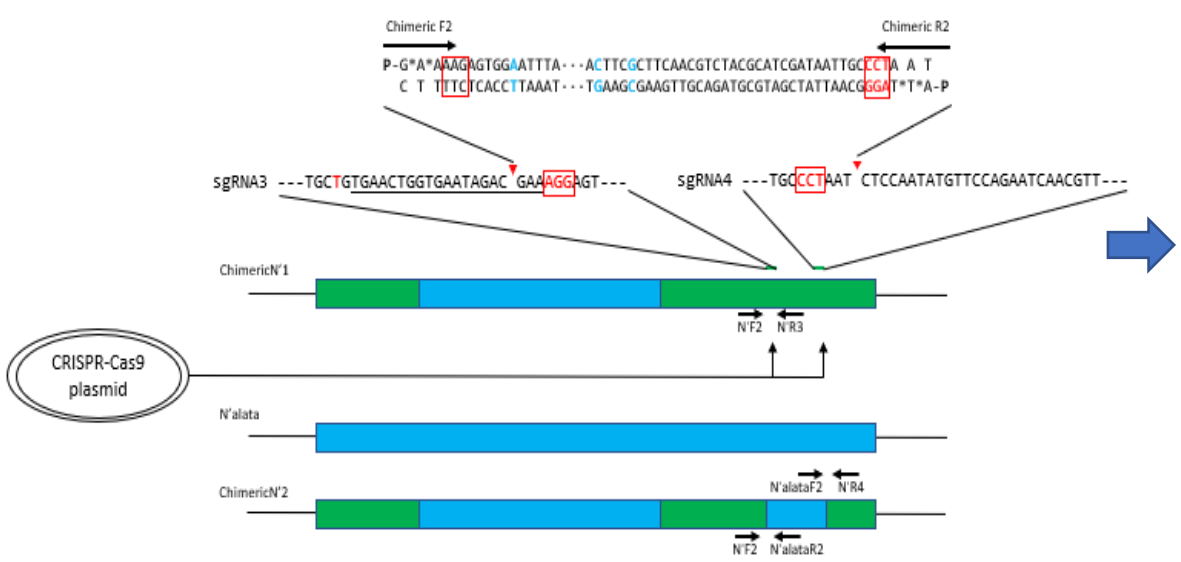

Non-Homologous End Joining (NHEJ)



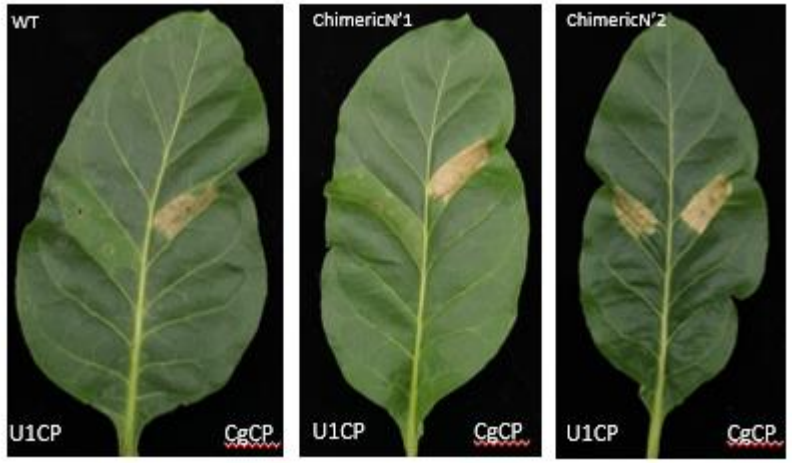




For the 1.8k fragment, the insertion ratio is ~5%, but the seamless insertion is ~0.25%.

# Gene replacement of *N'* with *N'alata* fragment








For the 400bp fragment, the insertion ratio is ~10%, the seamless insertion is ~1%.

Edited K326 gained TMV resistance

# Acknowledgement

## Our group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# Thanks for your attention!