Journal Club

Cell

Article

All-RNA-mediated targeted gene integration in mammalian cells with rationally engineered R2 retrotransposons

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[4]Bejing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China
[5]Northeast Agricultural University, Harbin 150030, China
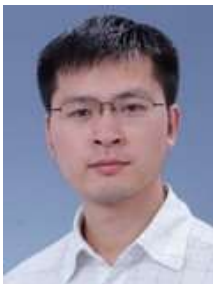[6]These authors contributed equally
[7]Lead contact
*Correspondence: zhouqi@ioz.ac.cn (Q.Z.), liwei@ioz.ac.cn (W.L.)
https://doi.org/10.1016/j.cell.2024.06.020

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教育与工作经历：

- 2006年获武汉大学理学学士学位
- 2012年获中国科学院大学理学博士学位
- 2013年入选中国科学院动物研究所"星辰研究员"计划并建立课题组
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研究方向：

- 基因工程和干细胞的创新生物技术研究
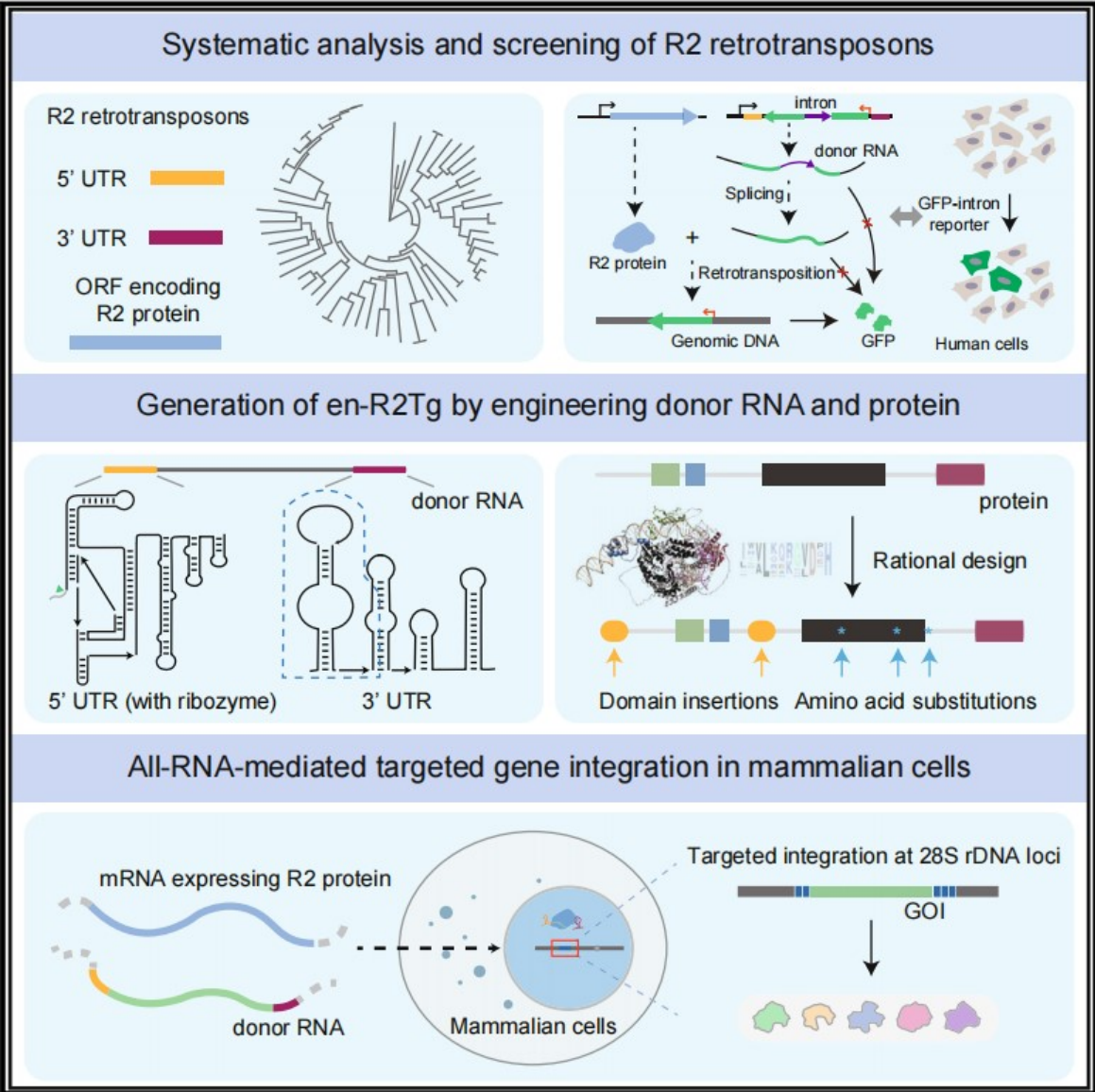- 开发基因治疗和疾病模型新技术

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教育与工作经历：

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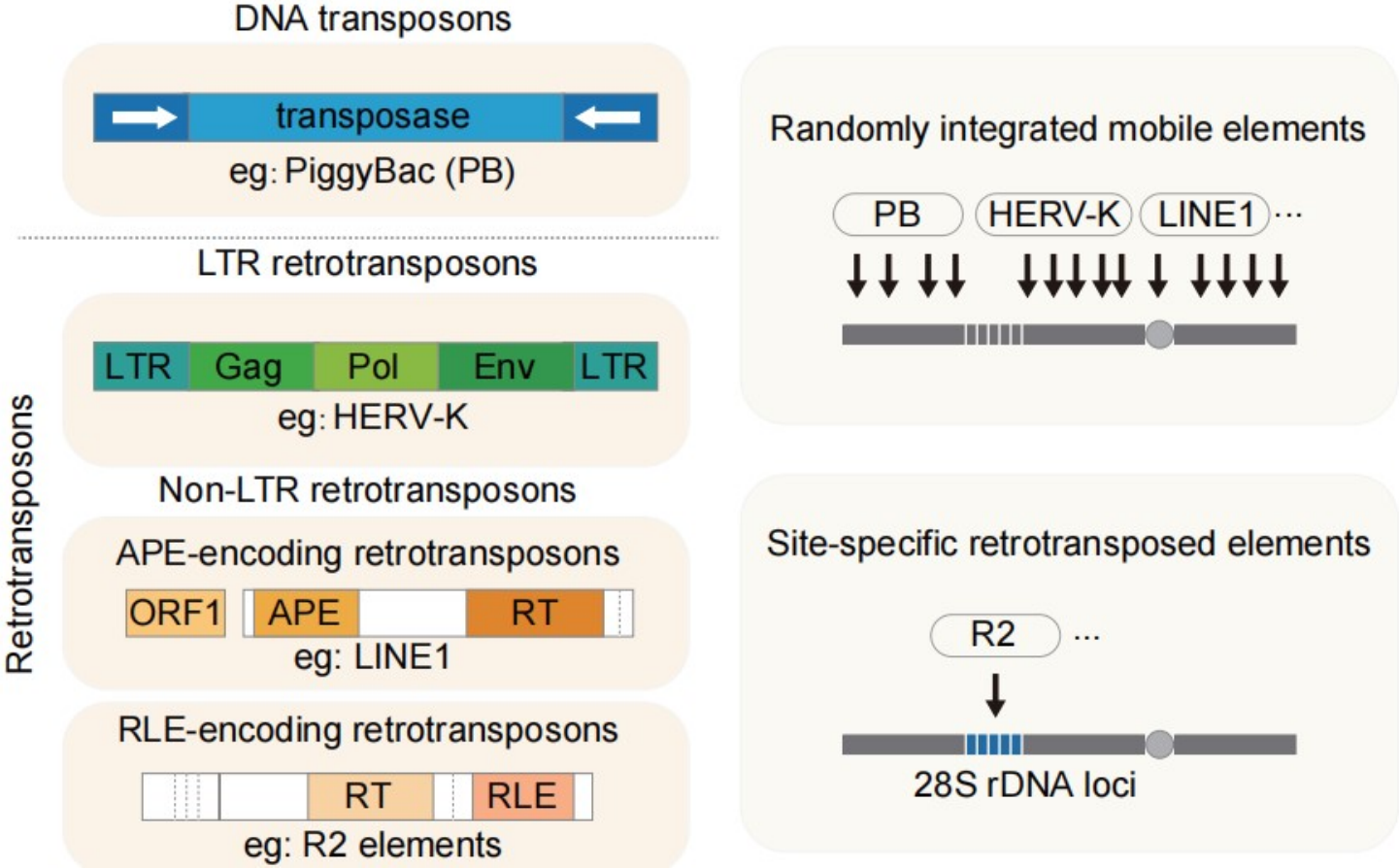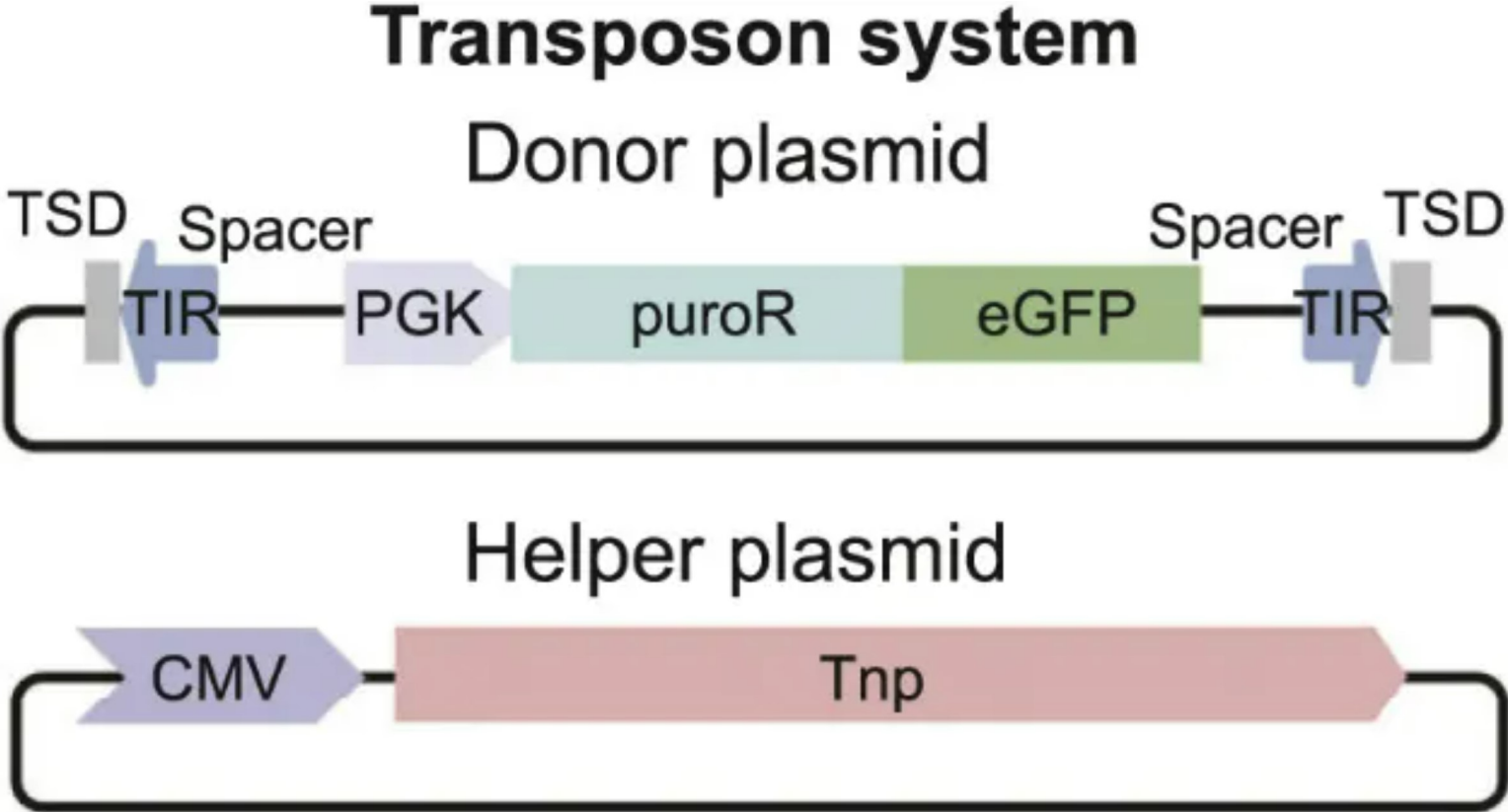- 领导国家重大科学研究计划、中国科学院干细胞领域战略性科技先导专项等重大项目

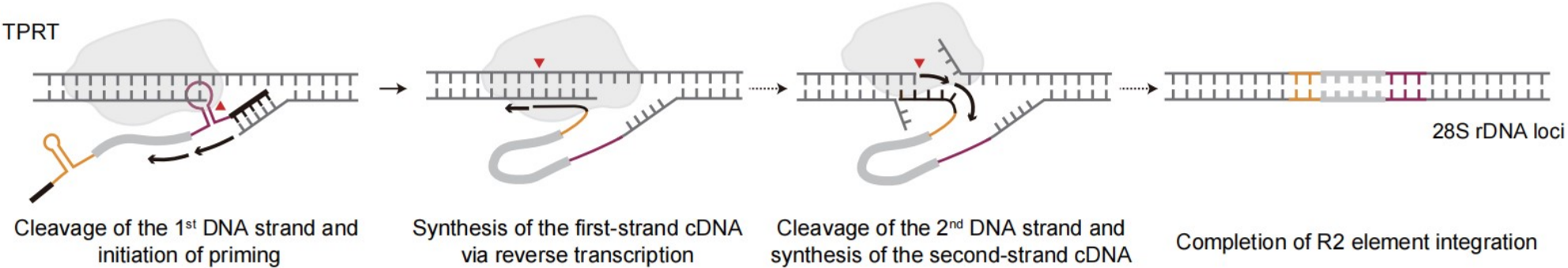**研究方向：**

- 干细胞与再生医学
- 干细胞应用研究与转化

Article overview

Systematic analysis and screening of R2 retrotransposons

R2 retrotransposons
5' UTR
3' UTR
ORF encoding R2 protein

intron
donor RNA
Splicing
R2 protein
Retrotransposition
Genomic DNA
GFP
GFP-intron reporter
Human cells

Generation of en-R2Tg by engineering donor RNA and protein

donor RNA
5' UTR (with ribozyme)
3' UTR

protein
Rational design
Domain insertions
Amino acid substitutions

All-RNA-mediated targeted gene integration in mammalian cells

mRNA expressing R2 protein
donor RNA
Mammalian cells
Targeted integration at 28S rDNA loci
GOI

筛选

改造

功能验证

Overview of Transposons

R2 is a site-specific retrotransposon targeted only 28S rDNA.

Typical gene integration system disigned from DNA transposons.

Transposon system

Donor plasmid

TSD Spacer — TIR — PGK — puroR — eGFP — TIR — Spacer TSD

Helper plasmid

CMV — Tnp

Advantages of R2 to target gene integration

- Mature and efficient ssRNA delivery technology.

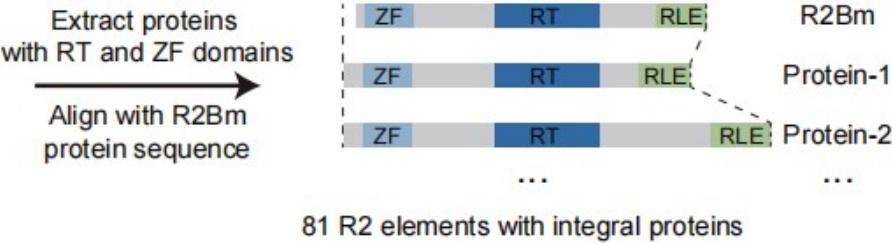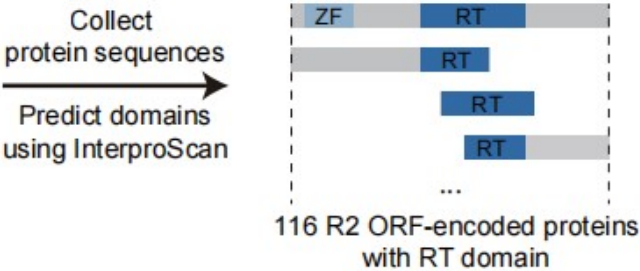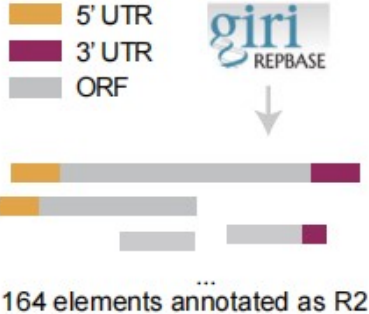- Safety (target 28S rDNA, does not disturb functional mRNAs)

Cleavage of the 1st DNA strand and initiation of priming

Synthesis of the first-strand cDNA via reverse transcription

Cleavage of the 2nd DNA strand and synthesis of the second-strand cDNA

Completion of R2 element integration
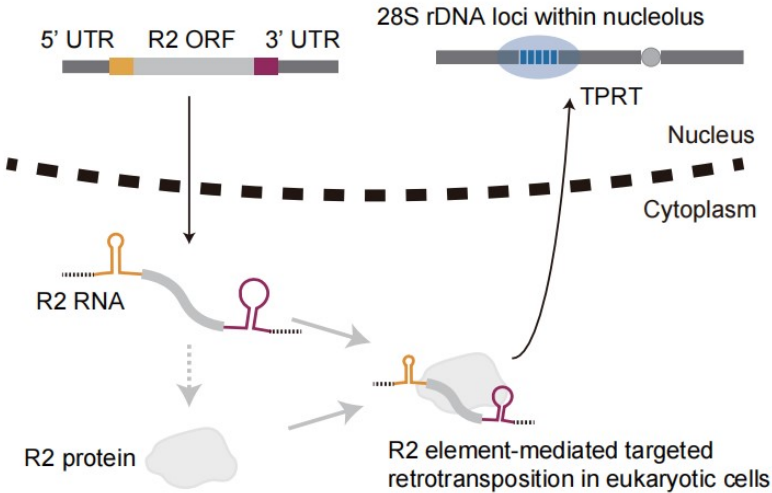
Study Objectives

- Systematic R2 retrotransposons analysis

- Engineering R2 elements for enhanced integration

- Demonstrating all-RNA-mediated targeted integration in mammalian cells
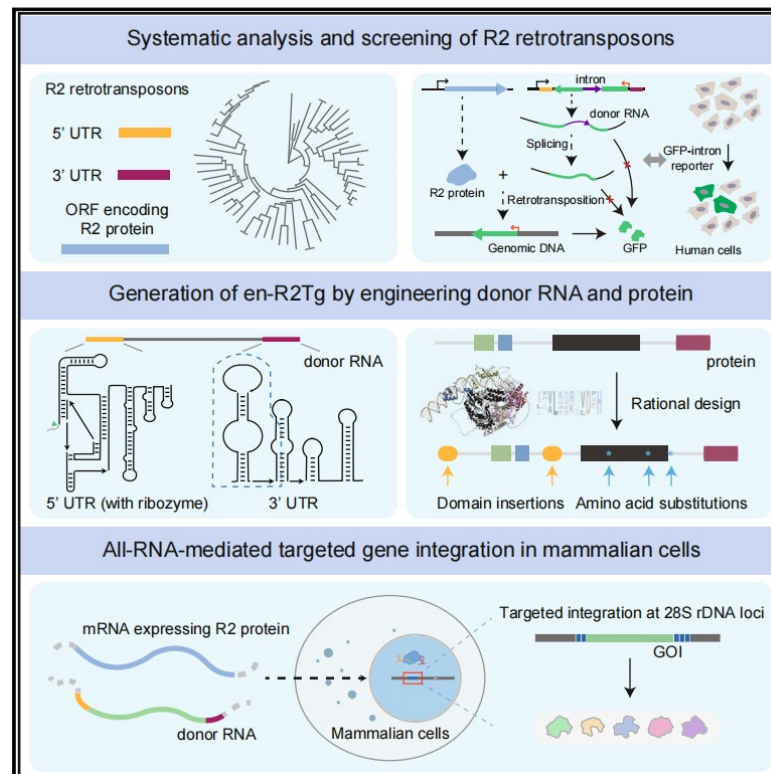
Bioinformatcs screened 62 complete R2 (integral for transposition)
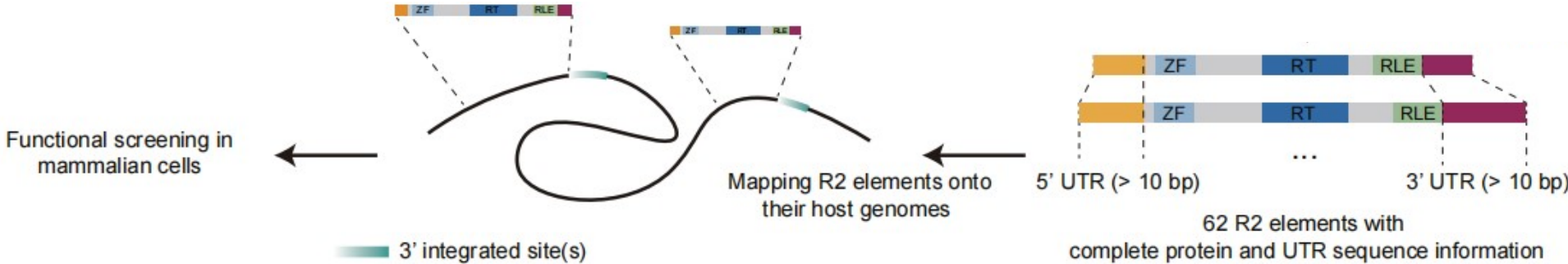
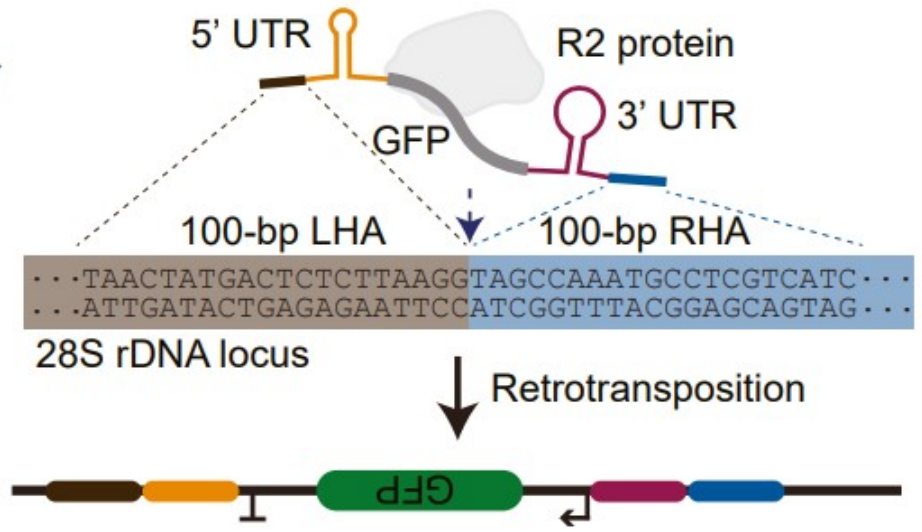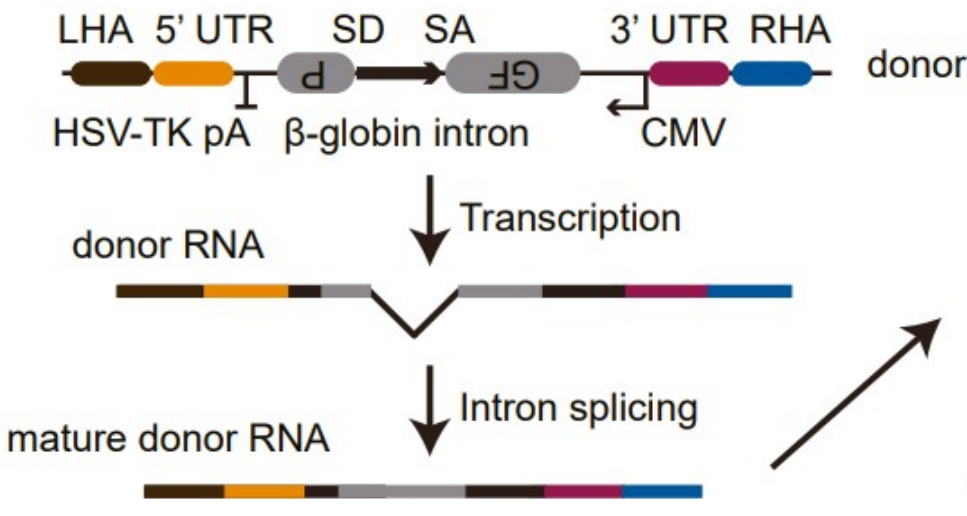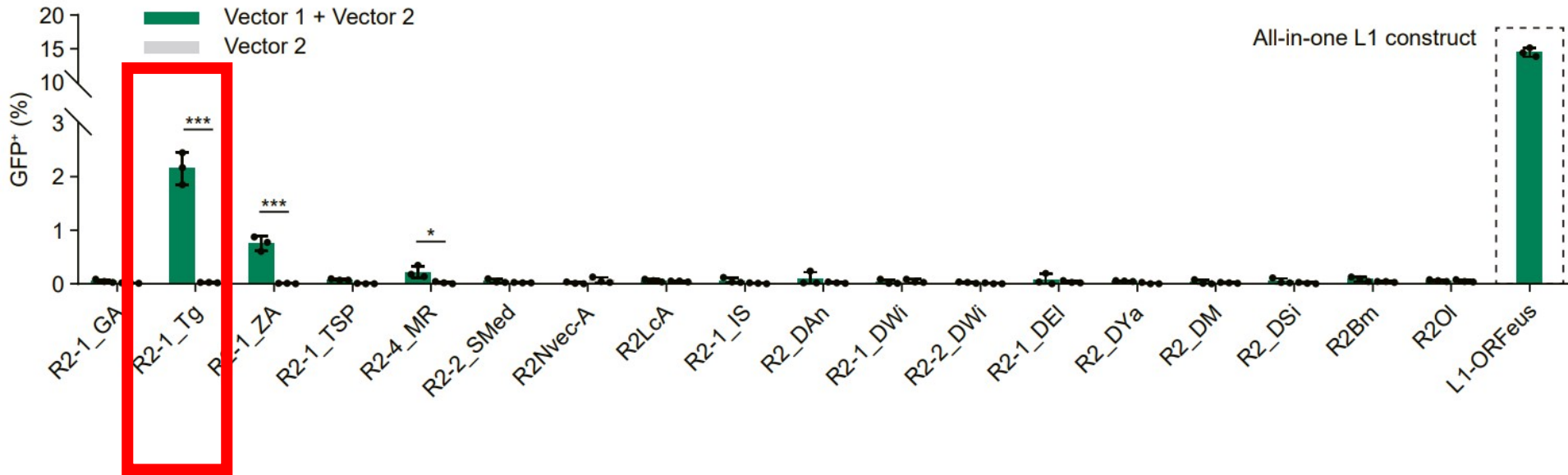# Modular expression system
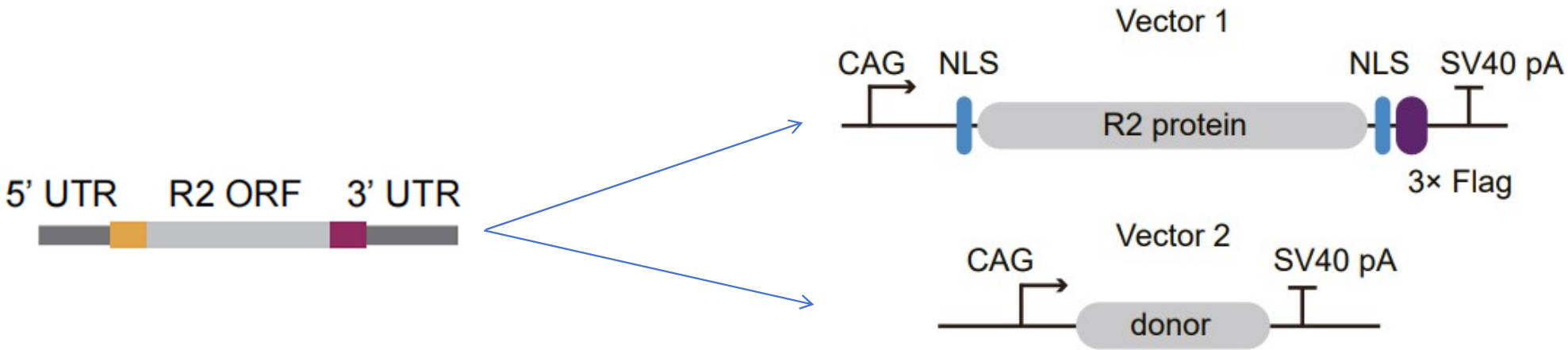
GFP-intron reporter system
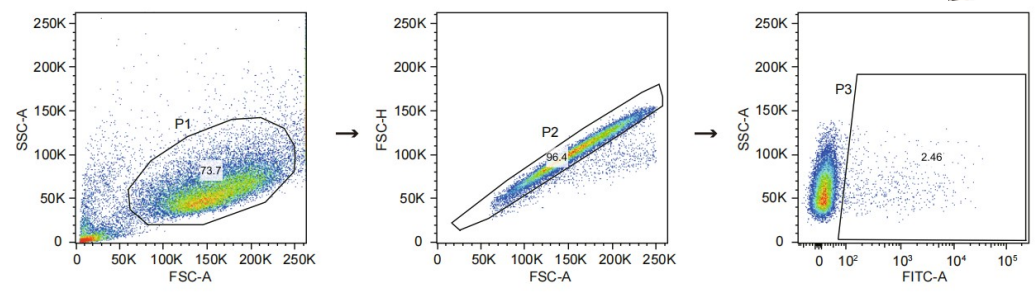
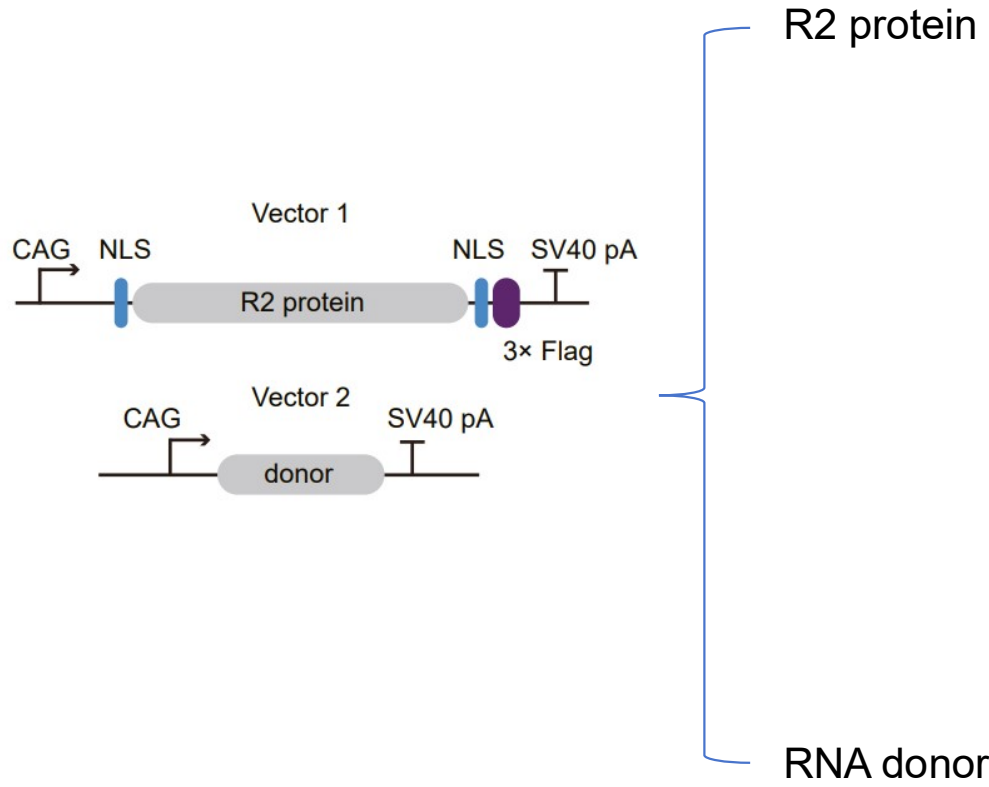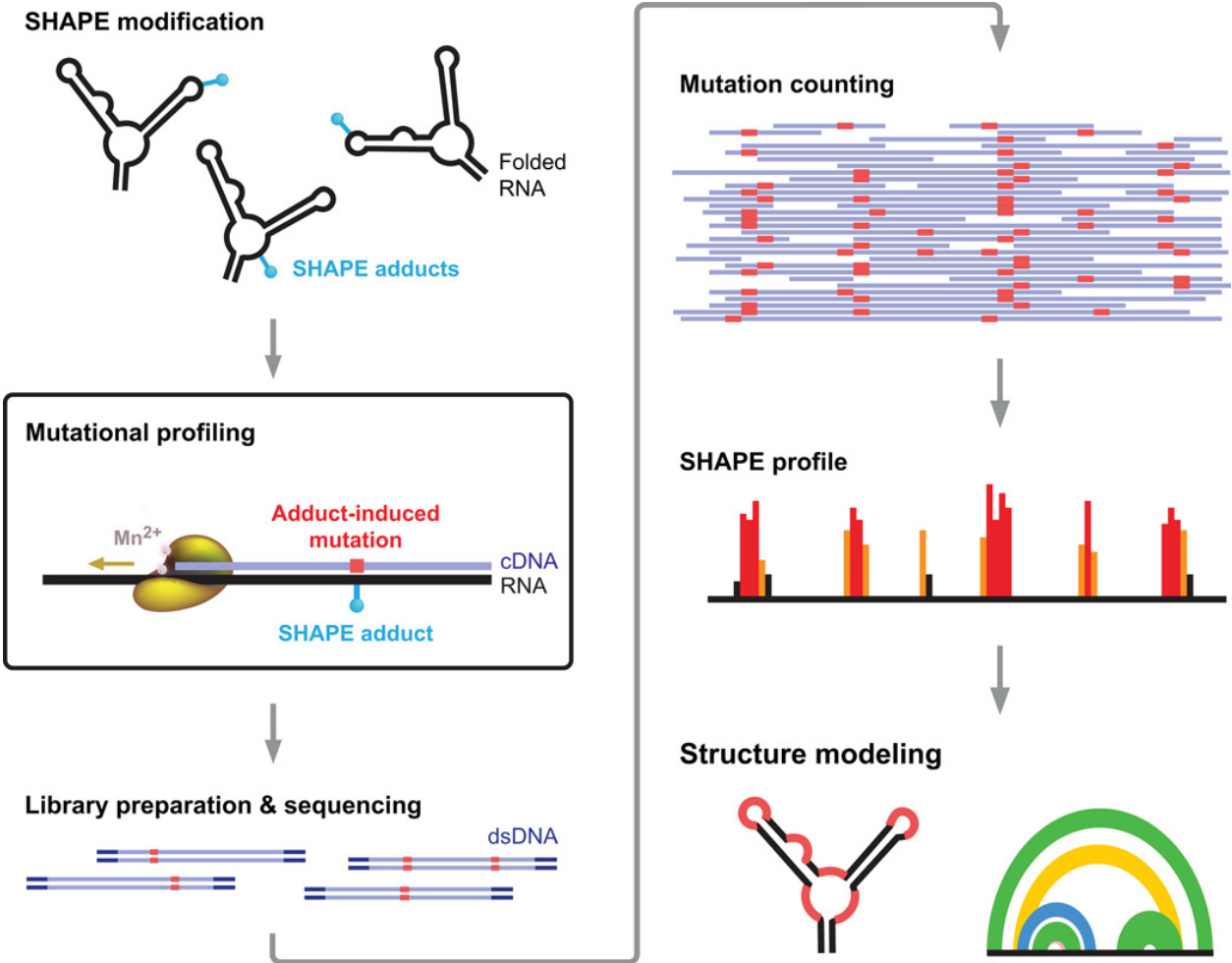# R2Tg has optimal retrotransposition activity

An, W. et al. Characterization of a synthetic human LINE-1 retrotransposon ORFeus-Hs. Mobile DNA, 2011.
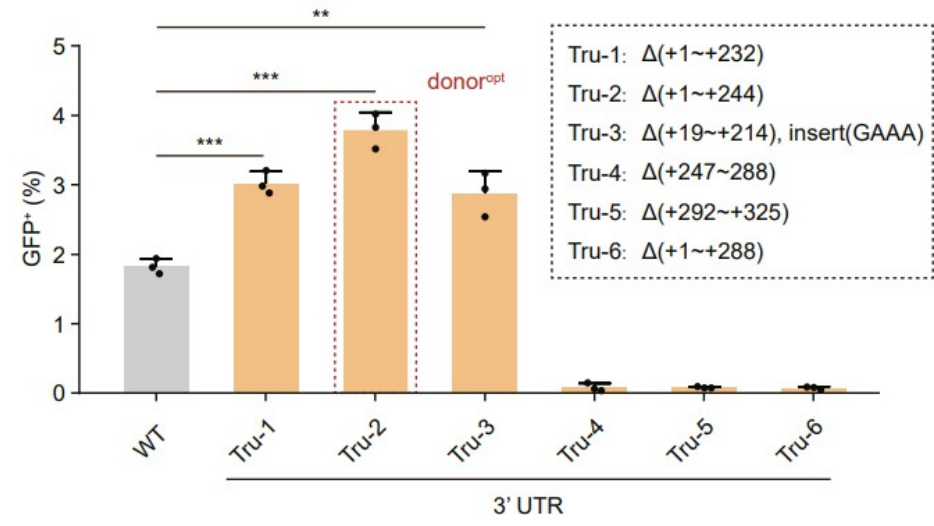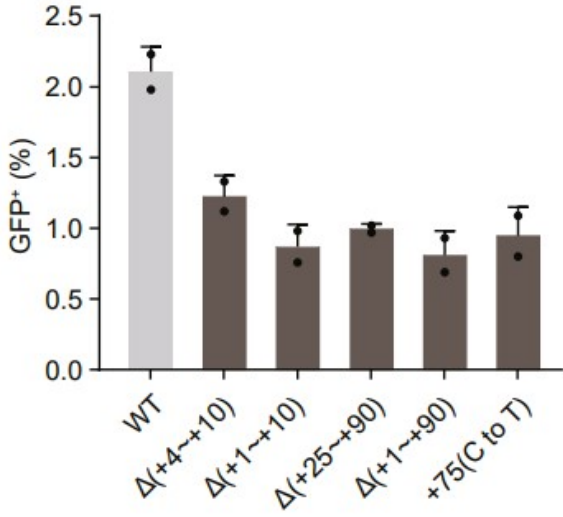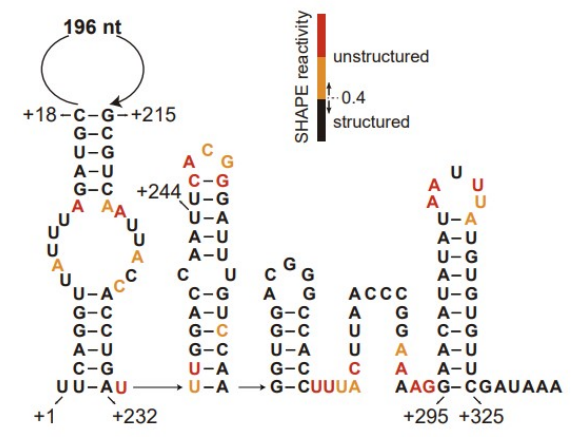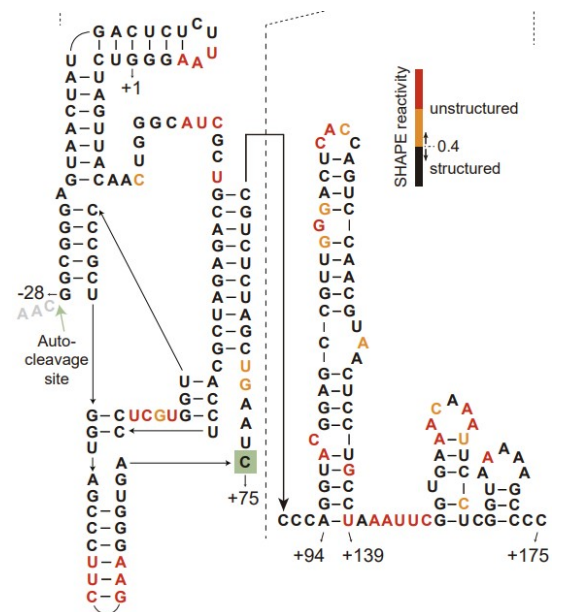
Engineering strategy
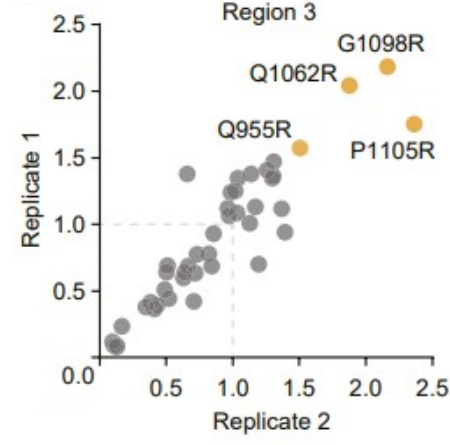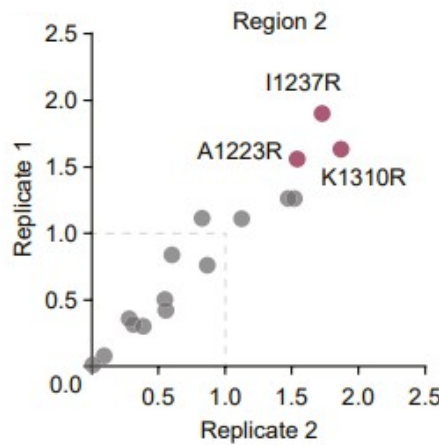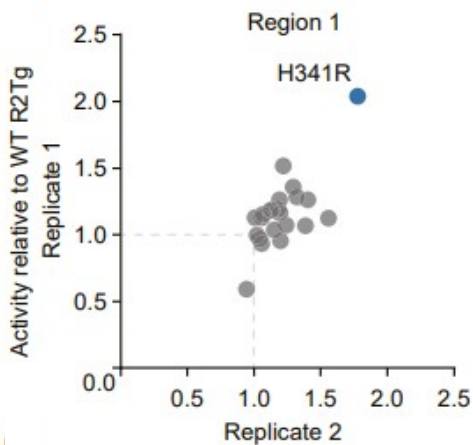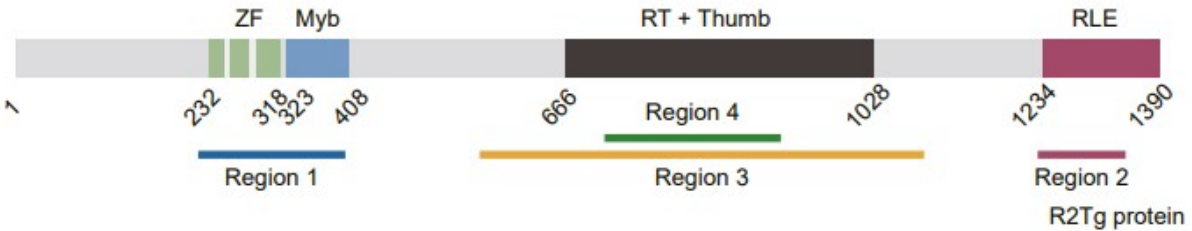
RNA structure determined by SHAPE-Map

Siegfried NA et al., "RNA motif discovery by SHAPE and mutational profiling (SHAPE-MaP)," Nat Methods, 2014.

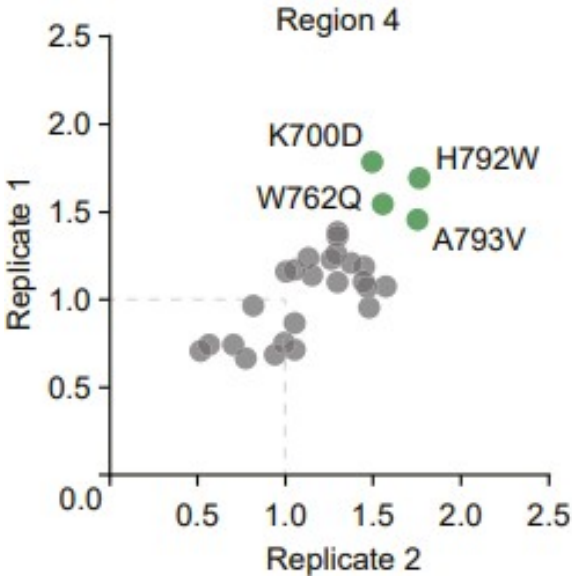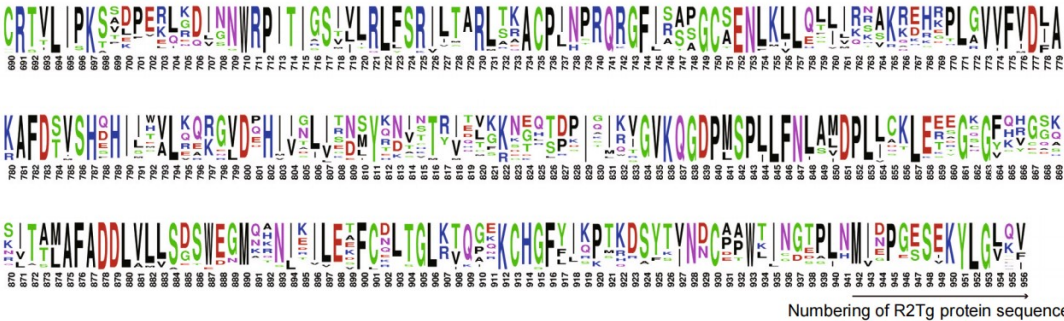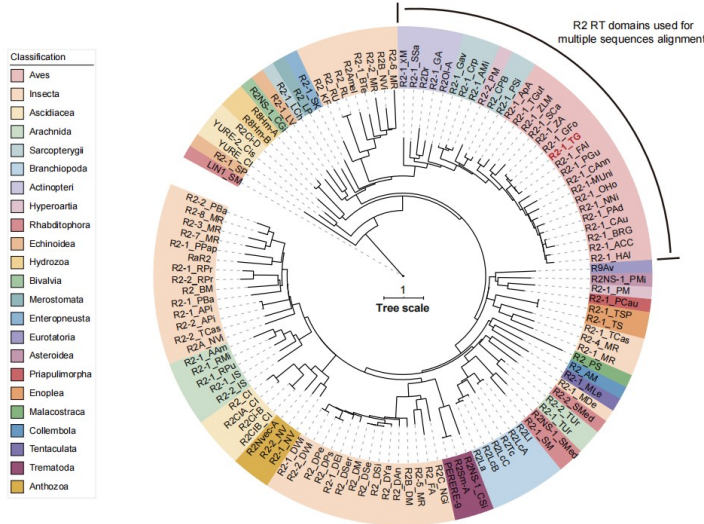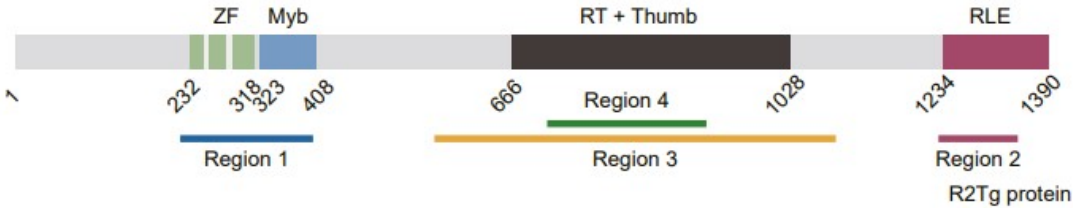# Truncation of 3'UTR (Tru-2, donor^opt) enhances integration.

Point mutation in R2 protein

Strategy 1. Arginine with positive charge enhances DNA binding ability.

Point mutation in R2 protein

R2 RT domains used for multiple sequences alignment

Classification

- Aves
- Insecta
- Ascidiacea
- Arachnida
- Sarcopterygii
- Branchiopoda
- Actinopteri
- Hyperoartia
- Rhabditophora
- Echinoidea
- Hydrozoa
- Bivalvia
- Merostomata
- Enteropneusta
- Eurotatoria
- Asteroidea
- Priapulimorpha
- Enoplea
- Malacostraca
- Collembola
- Tentaculata
- Trematoda
- Anthozoa

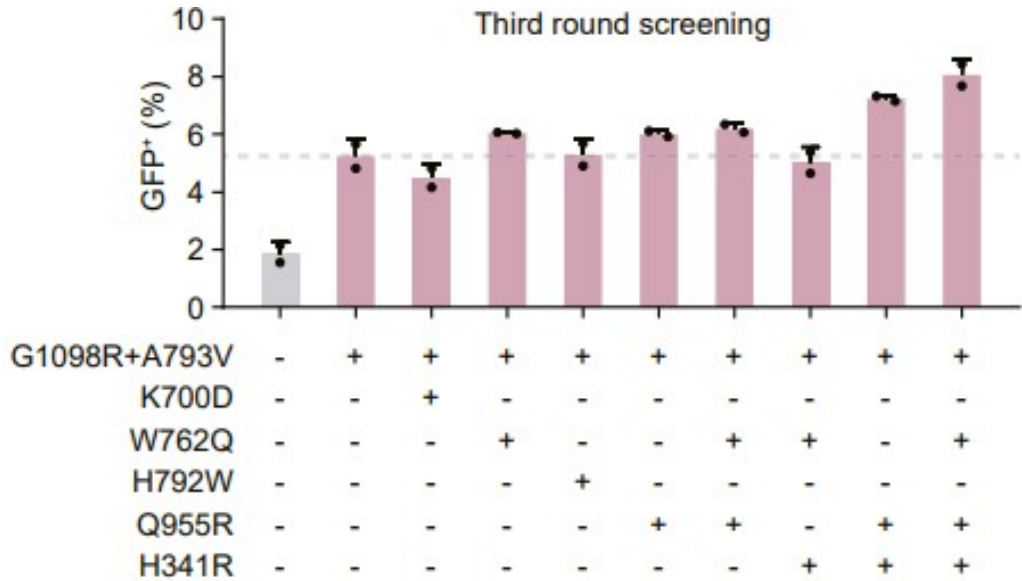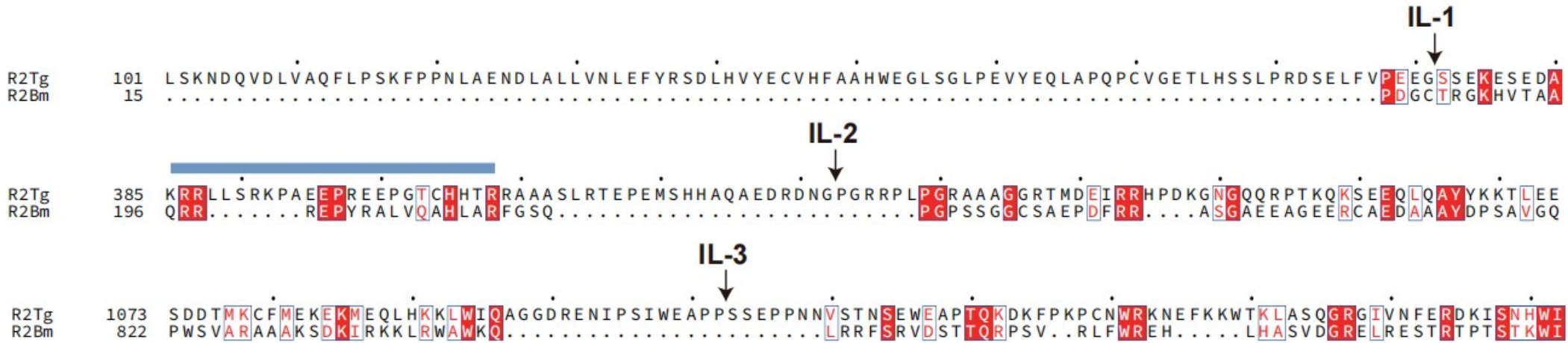Numbering of R2Tg protein sequence

Region 4

Strategy 2. Integrate consensus amino acids of RT domain.

Point mutation in R2 protein

Second round screening

Third round screening

G1098R+A793V	-	+	+	+	+	+	+	+	+	+
K700D	-	-	+	-	-	-	-	-	-	-
W762Q	-	-	-	+	-	-	+	+	-	+
H792W	-	-	-	-	+	-	-	-	-	-
Q955R	-	-	-	-	-	+	+	-	+	+
H341R	-	-	-	-	-	-	-	+	+	+
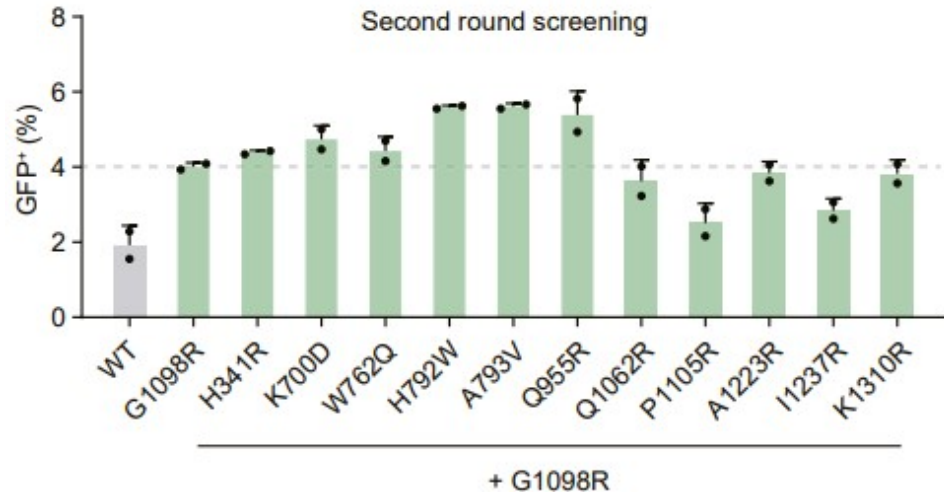
Domain insertion in R2 protein

Domain insertion in R2 protein

Activity relative to WT R2Tg

Combination of domain insertions
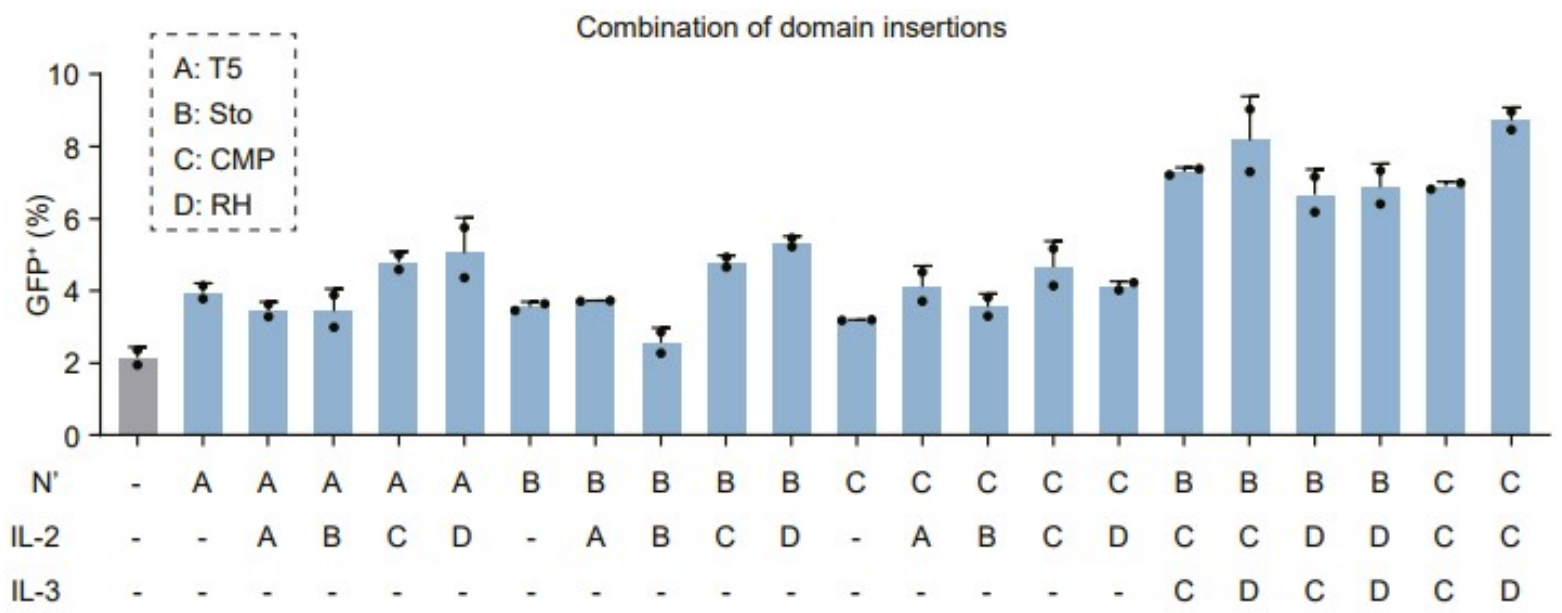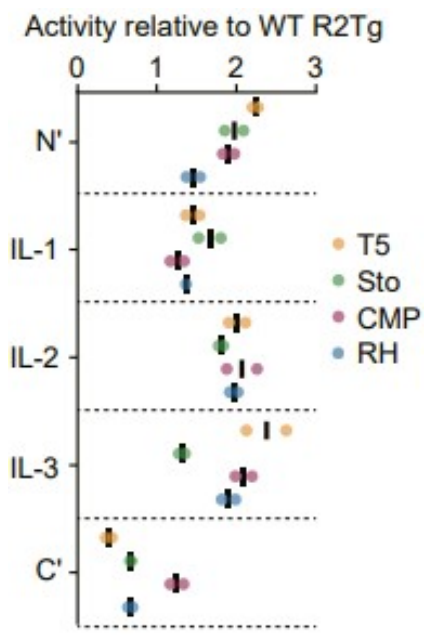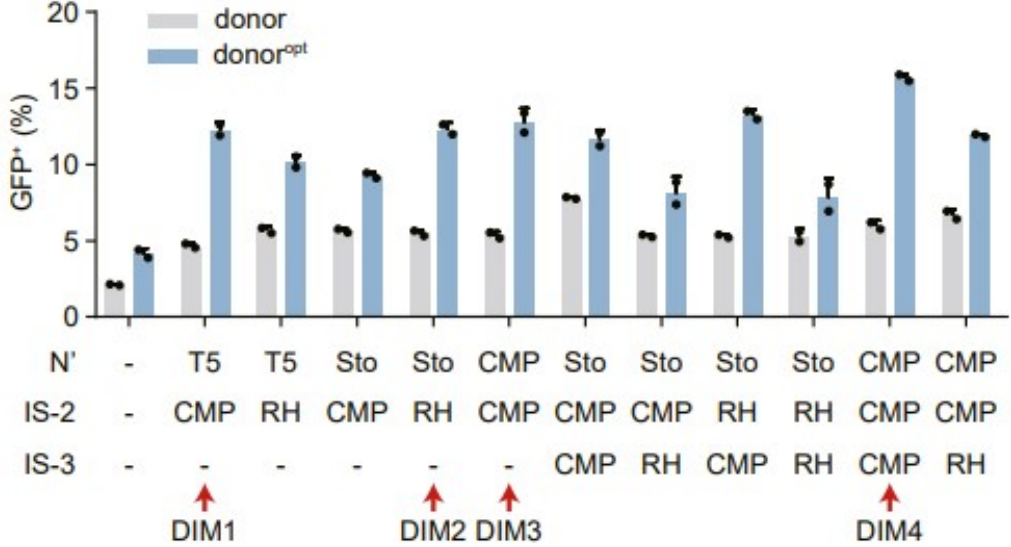
A: T5
B: Sto
C: CMP
D: RH

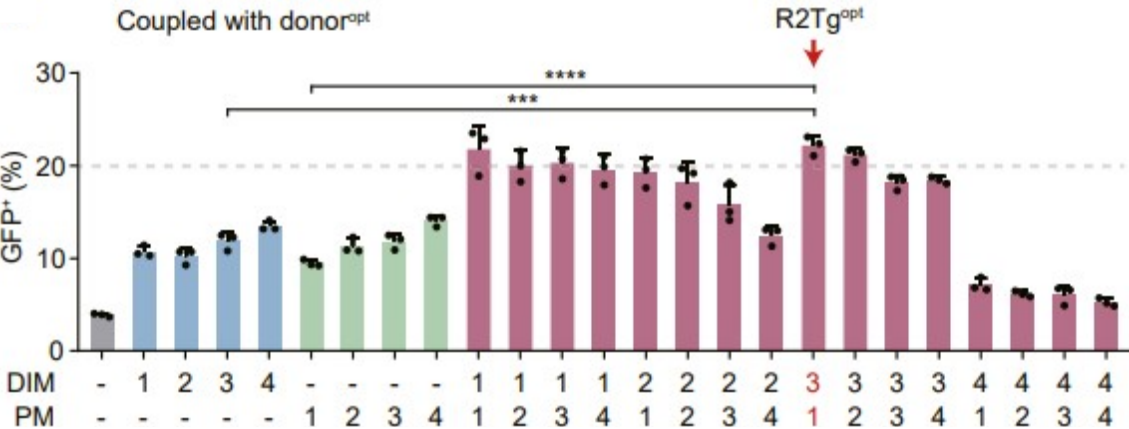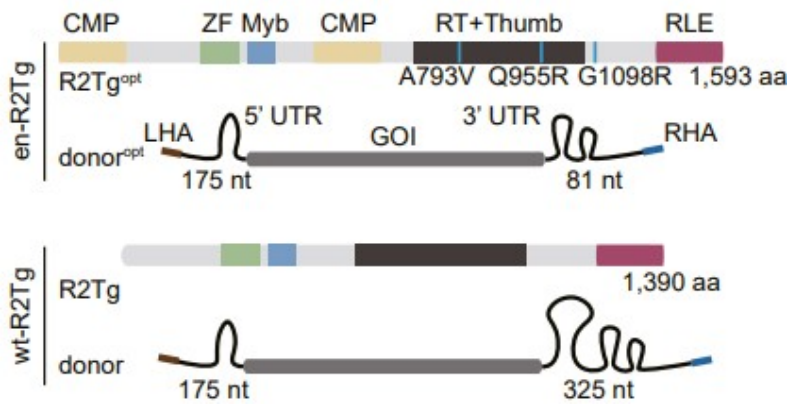| N'	-	A	A	A	A	A	B	B	B	B	B	C	C	C	C	C	B	B	B	B	C	C
IL-2	-	-	A	B	C	D	-	A	B	C	D	-	A	B	C	D	C	C	D	D	C	C
IL-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	D	C	D	C	D
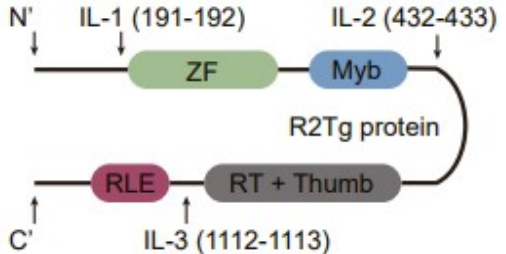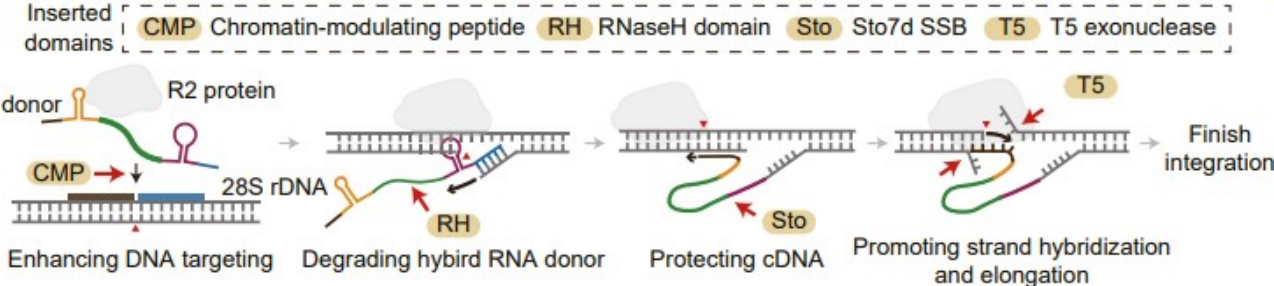
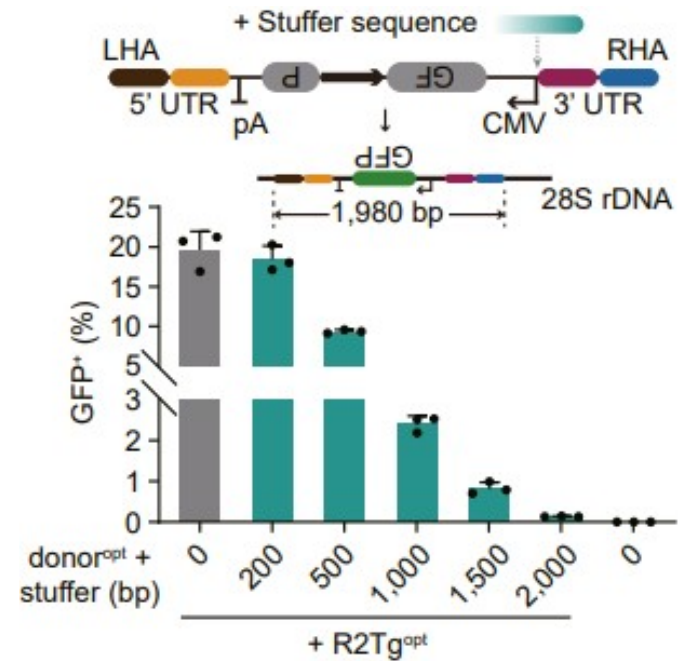Engineered RNA donor and R2 protein enhances integration synergisticly.
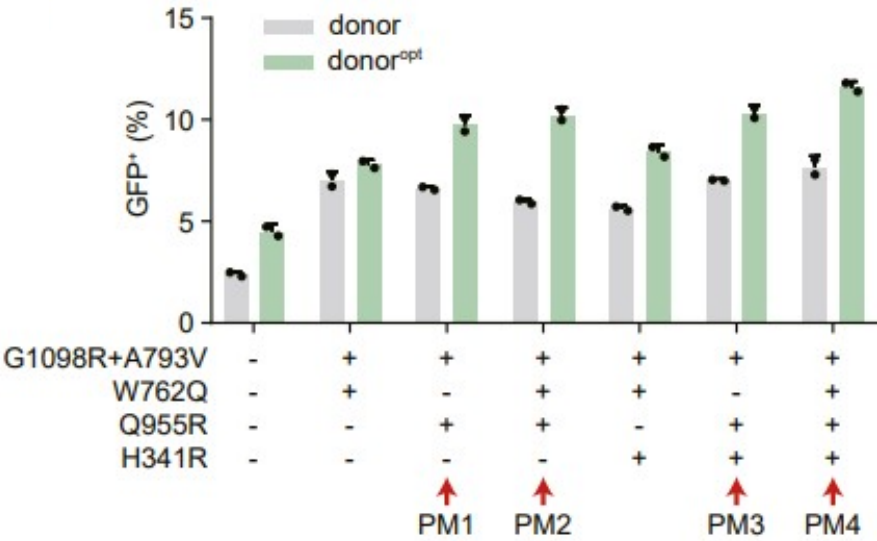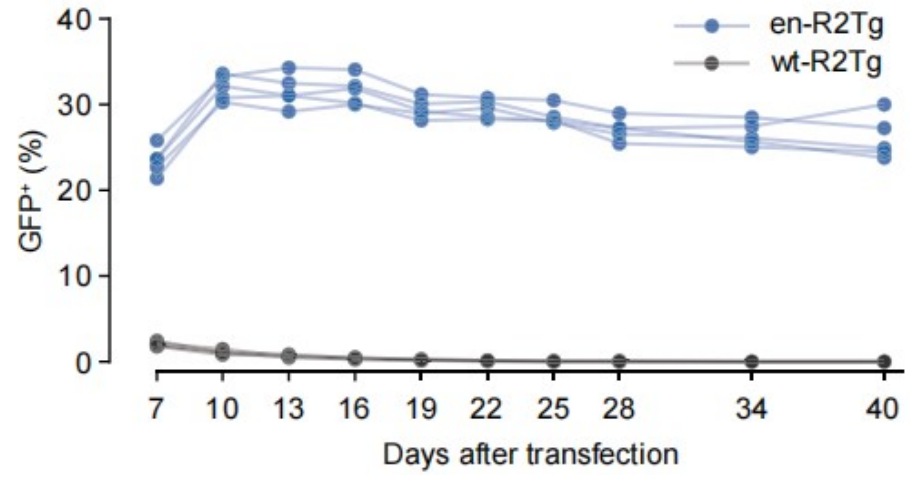
Combination of strategies generated en-R2Tg

CMP(N)-CMP(IL-2)-R2Tg(A793V/Q955R/G1098R)
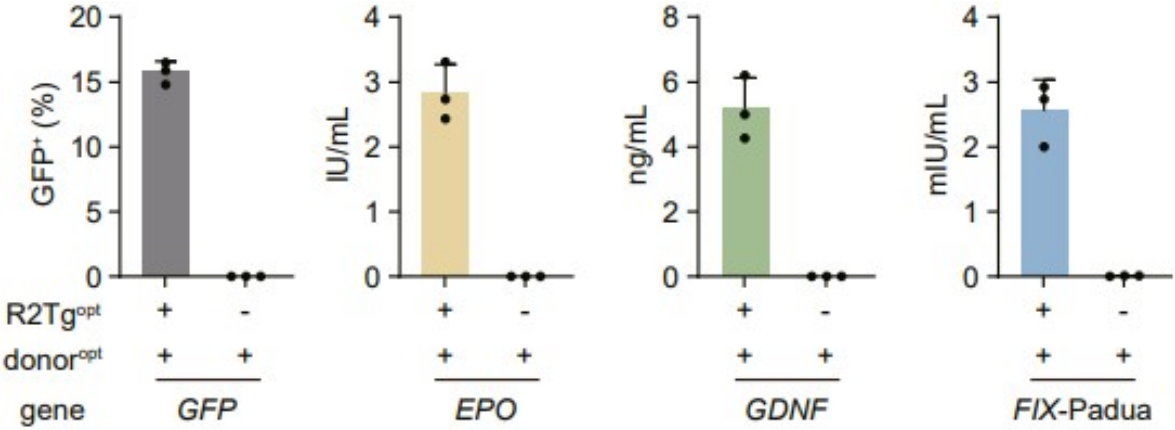
Bigger than 2.5kb insertion capacity.
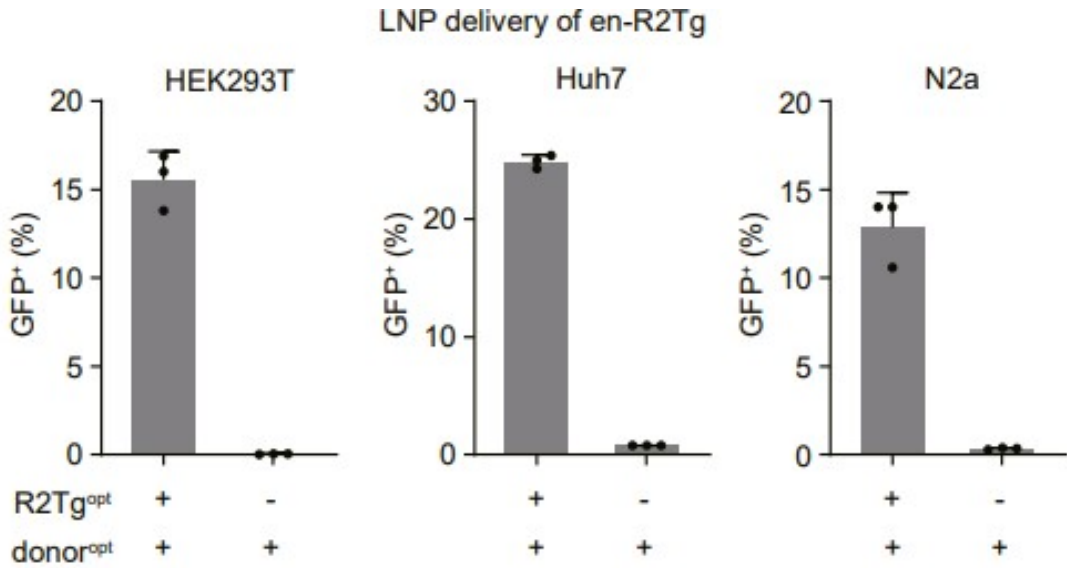
Stable expression over 1 month.

(in HEK293T cells by extended cell culture)
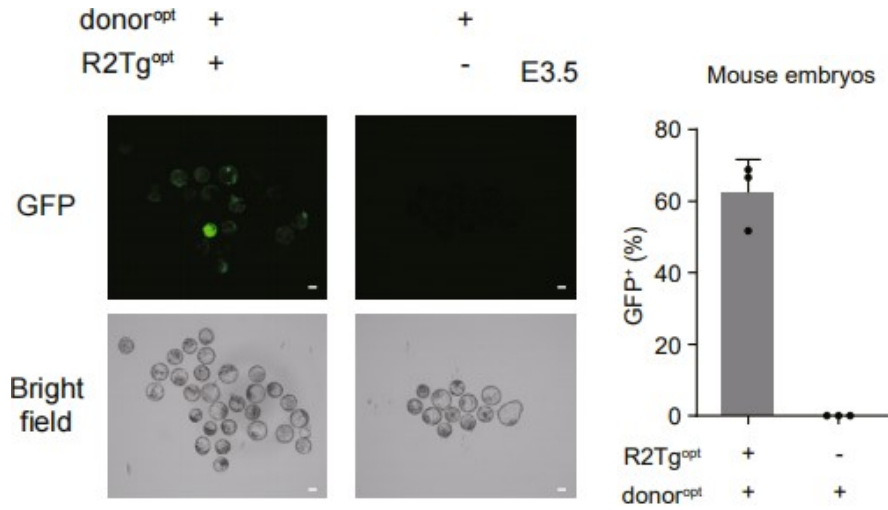
Integrate different genes in HEK293T.

In-vitro transcribed RNA, delivered by Lipo3000.

Examples in multiple cell lines and zygote cells.

LNP delivery of en-R2Tg

HEK293T

GFP^+ (%)

| R2Tg^{opt} | + | - |
| donor^{opt} | + | + |

Huh7

GFP^+ (%)

| | + | - |
| | + | + |

N2a

GFP^+ (%)

| | + | - |
| | + | + |

Microinjection of RNAs into mouse zygotes

R2 protein mRNA — AAAAA
donor RNA — AAAAA

| donor^{opt} | + | + |
| R2Tg^{opt} | + | - | E3.5 |

GFP

Bright field

Mouse embryos

GFP^+ (%)

| R2Tg^{opt} | + | - |
| donor^{opt} | + | + |
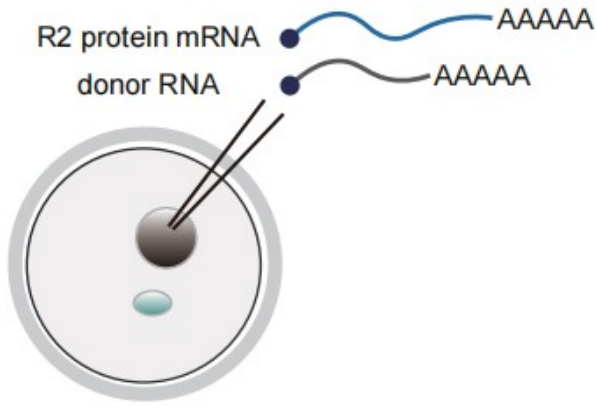
Precise integration specificity at 28S rDNA.
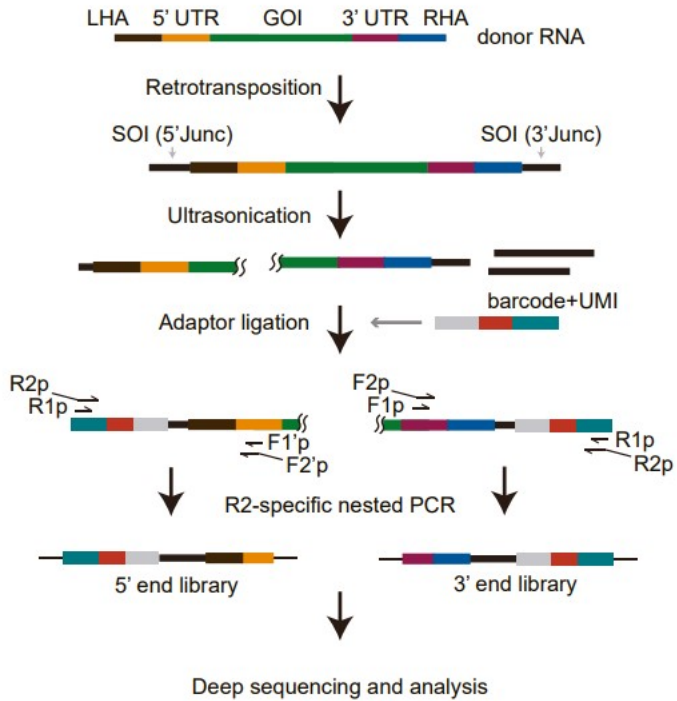
89.8% of 5' junction reads have exact integration sites

73.2% (en-R2Tg) and 74.2% (wt-R2Tg) of these reads contained the following configuration

Other regions of rDNA or genome
28 bp LHA
5' UTR

Auto-cleavage site of R2Tg ribozyme
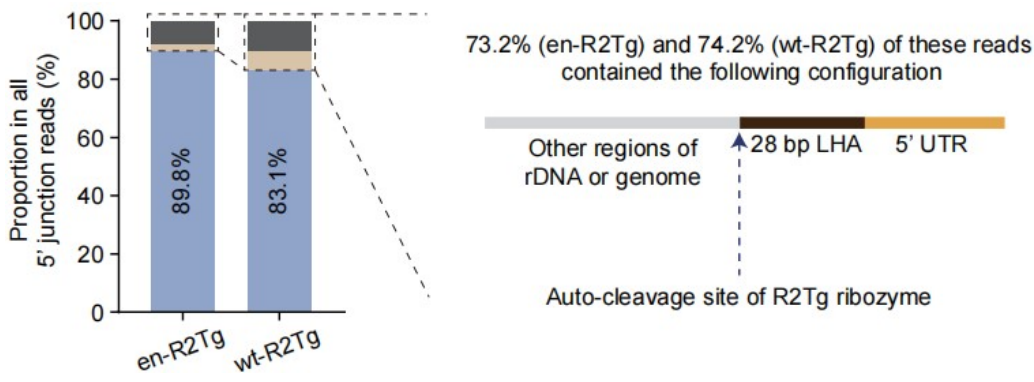
99.4% of 3' junction reads have exact integration sites

On-target integration
Off-target integration
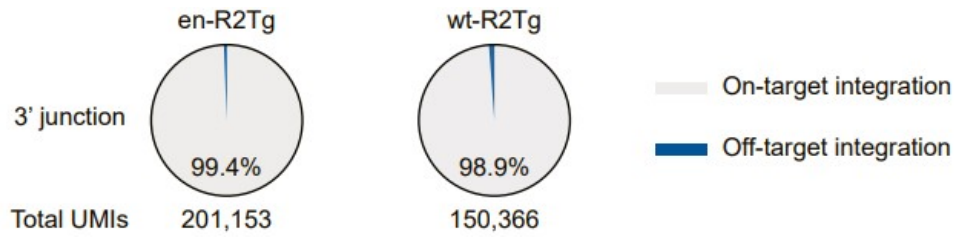
Capturing retrotransposon-integrated transgene sequencing (CRIT-seq)

Models explain the differences between 5' and 3' junction precision

rDNA loci

Template jumping ?

cDNA of transgene

Cellular RNAs ?
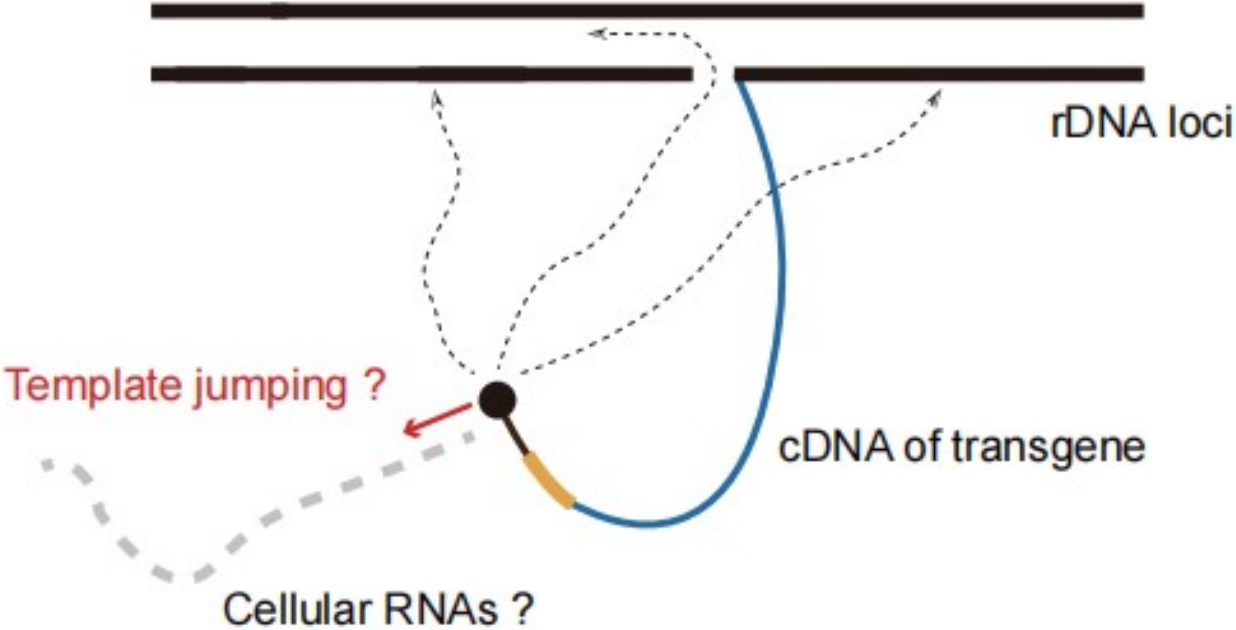
Summary

- Systematic analysis and screening of naturally occurring R2 retrotransposons.

- Engineering of donor RNA and protein enhances R2-mediated gene insertion activity.

- All-RNA-delivered en-R2Tg enables effective gene integration in mammalian cells.

- Optimized en-R2Tg system has high on-target integration specificity in genome.