Supplementary Information for

Interfering with retrotransposition by two types of CRISPR effectors: Cas12a and Cas13a

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CONTENTS:

SUPPLEMENTARY FIGURES

Figure S1

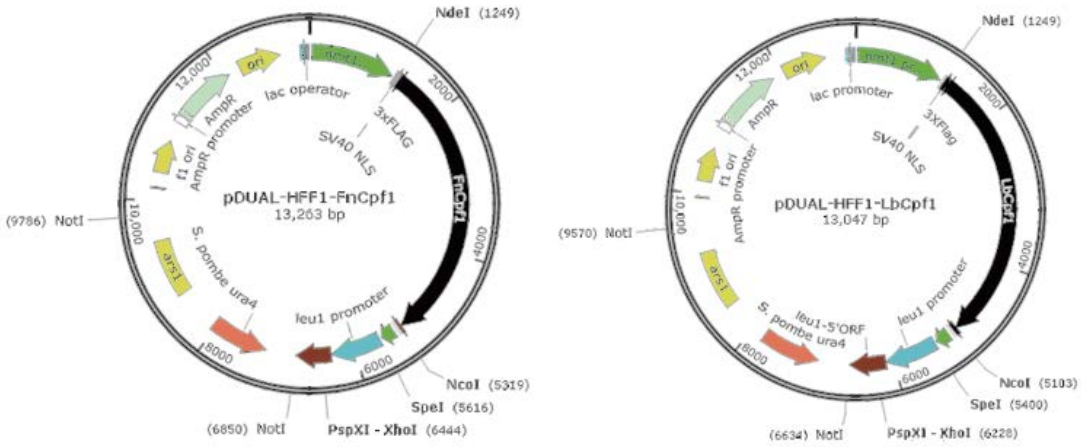


Figure S1. Maps of plasmids of pDUAL-HFF1-FnCpf1 and pDUAL-HFF1-LbCpf1 for chromosome-integrated expression of FnCpf1 and LbCpf1 in *S. pombe*. The FnCpf1 and LbCpf1 expression cassettes contain the *nmt*1 promoter and the *ADH*1 terminator. The SV40-derived nuclear localization signal (NLS) was fused to both N and C termini of Cpf1; a 3 × Flag was in-frame fused to the N terminus of NLS.

Figure S2

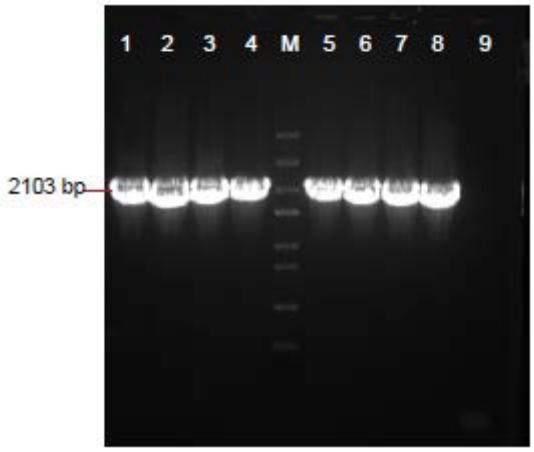
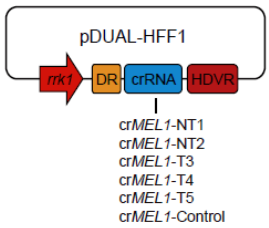


Figure S2. Diagnostic PCR for *S. pombe* transformants with FnCpf1 and LbCpf1 expression cassettes integrated into the chromosome. Primer set ADHterm-F/leu1-R (Supplementary Data 1) was used for diagnostic PCR. Lanes 1-4: *S. pombe* transformants with integrated expression construct of FnCpf1; Lanes 5-8: *S. pombe* transformants with integrated expression construct of LbCpf1; Lane 9: control wild-type *S. pombe* strain (without integrated expression constructs); M: *Trans*5K DNA Marker (Trans Transgen Biotech, Shanghai, China).

Figure S3

a



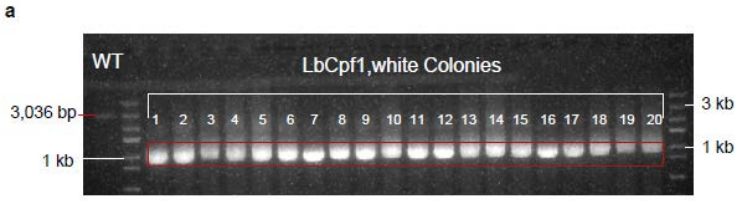
b

Target gene	crRNA name	PAM - guide sequence (5'-3')	Position from ATG	Gene editing efficiency	
				FnCpf1 chromosomal	LbCpf1 chromosomal
MEL1 (1311 nt)	cr*MEL1*-NT1	T(TTG)CAGATTGGGGAGTAGATTACTTAAA	431	71%	28%
	cr*MEL1*-NT2	T(TTC)CAATAATGATTGGGTAGTGGCTGTT	1050	29%	20%
	cr*MEL1*-T3	T(TTA)GTCCCAAACCATTATAGCTACCATG	67	77%	14%
	cr*MEL1*-T4	T(TTG)CTTCCAATCTACCAGTGGTTGCATT	250	45%	35%
	cr*MEL1*-T5	T(TTA)AGTAATCTACTCCCCAATCTGCAAA	427	77%	53%
	cr*MEL1*-Control	AACAGCGCCTTAAAAGAACTAGAAA		0	0

Figure S3. Editing on *MEL1* gene by chromosomal expressing FnCpf1 and LbCpf1 with different crRNAs. (a) Schematic representation the crRNA expression cassette for FnCpf1 and LbCpf1. *rrk1*, *rrk1* promoter; DR, direct repeat of crRNA; HDVR, hepatitis delta virus ribozyme. (b) Targeting sequences and editing efficiency on *MEL1* gene by chromosomal expressing FnCpf1 or LbCpf1 with five different crRNAs in *S.pombe*. The PAM motif (TTTN for FnCpf1 and LbCpf1) regions are underlined.

Figure S4

a



b

cr*MEL1*-T5 _Col1:

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CGAAAAATTTTTTTAAAAAAGTTTATTATTATTTTTTTCTGTACATCAAAATATTCAACGCTAAGAAAACCATAGAAAAATGCG
CATCAAAATTTTAATTAGGATATAGCAGATGCTATAACAAAAGGATTTGTCTAAAACCTAGAGATATTATTAAAGCTTTTCTAA
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cr*MEL1*-T5 _Col2:

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ATTGCACGCCAGTTT

c

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TGCCTATTATATCATCTGACGCACTTGGAGCTGTACCACGTTTGGTTAGCTTCCCATTTTATTGAATACTGAGAATTGCAACAATCT
CTTATATTTATAATGCTCGATGTCCTGTTAACGAAAAATAAAGTATTTAAATGGATTGAGTAAAAATGAACCGTAAAGATTTGTAAT
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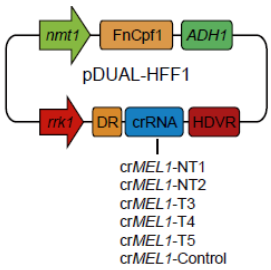
ACTAACAAATTTTATTAAATTCGGGGCTCGTTCTACTATAGC

Figure S4. Diagnostic PCR products and sequencing of *mel1* mutants generated by LbCpf1 editing. (a) Diagnostic PCR products of white colonies of *mel1* mutants generated by LbCpf1. All colonies show a 998-bp band indicating deletion of *MEL1* from LbCpf1 editing. The wild type strain shows a 3036-bp band. The primer set used for PCR were described in Figure 1c. (b) Sequences of PCR products from two randomly selected *mel1* mutant colonies. The sequence results show the correct deletion of the center portion of *MEL1* sequence. The blue and yellow highlighted sequences represent the upstream and downstream regions of *MEL1* gene, whereas the central potion is deleted. (c) Sequence of the template which was used for editing of *MEL1* gene by FnCpf1 or LbCpf1 through homologous recombination (HR). Blue sequence, the left arm of the HR template, which was amplified using primers of MEL1-P5, mel1-left-p3-new from *S.pombe* genomic DNA. Yellow sequence, the right arm of the HR template, which was amplified using primers MEL1-P3 and mel1-right-P5-new from *S.pombe* genomic DNA. The HR template was obtained by over-lap PCR using primers MEL1-P5, MEL1-P3 from left and right arms.

Figure S5

a



b

Target gene	crRNA name	PAM - guide sequence (5'-3')	Position from ATG	Gene editing efficiency	
				FnCpf1 chromosomal	FnCpf1 episomal
MEL1 (1311 nt)	cr*MEL1*-NT1	T(TTG)CAGATTGGGGAGTAGATTACTTAAA	431	71%	29%
	cr*MEL1*-NT2	T(TTC)CAATAATGATTGGGTAGTGGCTGTT	1050	29%	59%
	cr*MEL1*-T3	T(TTA)GTCCCAAACCATTATAGCTACCATG	67	77%	67%
	cr*MEL1*-T4	T(TTG)CTTCCAATCTACCAGTGGTTGCATT	250	45%	38%
	cr*MEL1*-T5	T(TTA)AGTAATCTACTCCCCAATCTGCAAA	427	77%	46%
	cr*MEL1*-Control	AACAGCGCCTTAAAAGAACTAGAAA		0	0

Figure S5. Editing efficiency on *MEL1* gene by chromosomal-expressing FnCpf1 and episomal-expressing FnCpf1 with five different crRNAs. (a) Schematic representation of the FnCpf1 and crRNA expression cassette in multi-copy plasmid pDUAL-HFF1. *rrk1*, *rrk1* promoter; DR, direct repeat of crRNA; HDVR, hepatitis delta virus ribozyme. (b) Targeting sequences and editing efficiency on *MEL1* gene by chromosomal-expressing FnCpf1 and episomal-expressing FnCpf1 with five different crRNAs in *S.pombe*. The PAM motif (TTTN for FnCpf1) regions are underlined.

Figure S6

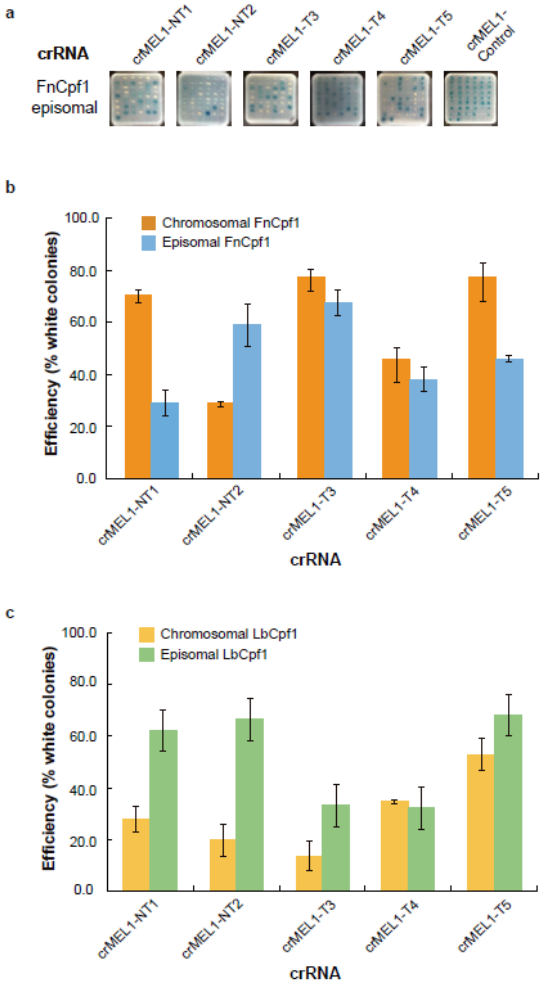


Figure S6. Editing of *MEL1* gene by chromosomal-expressing FnCpf1 and episomal-expressing FnCpf1 with five different crRNAs. (a) The editing on *MEL1* gene by episomal-expressing FnCpf1 with five different crRNAs, estimated by formation of white- (*mel1* mutant) and blue-colored (*MEL1* wildtype) colonies, assayed on agar plates containing the X-α-Gal. (b) Efficiency of genome editing on *MEL1* gene by chromosomal expressing FnCpf1 and episomal expressing FnCpf1 with five different crRNAs. Bar represents means ± s.e.m. with n = 3. (c) Efficiency of genome editing on *MEL1* gene by chromosomal expressing LbCpf1 and episomal expressing LbCpf1 with five different crRNAs. Bar represents means ± s.e.m. with n = 3.

Figure S7

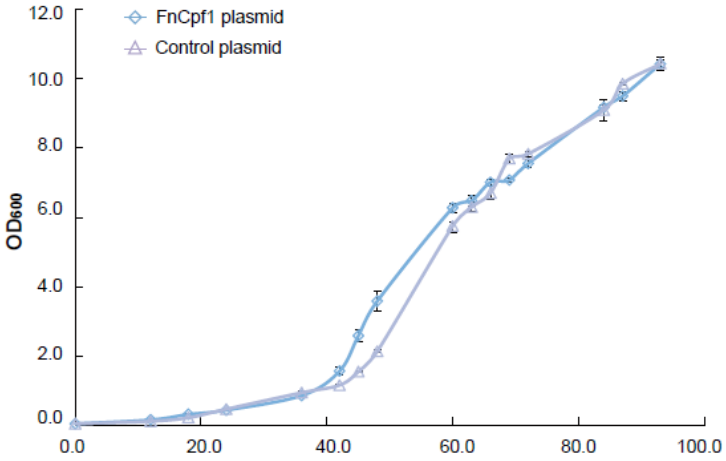


Figure S7. Growth in liquid culture media (EMM + uracil) of *S. pombe* strains with episomal expressing FnCpf1 compared to the control strain with control plasmid pDUAL-HFF1. They had an average double time of 9.2 h and 9.6 h, respectively. Data are means ± s.e.m. (n = 4 independent experiments)

Figure S8

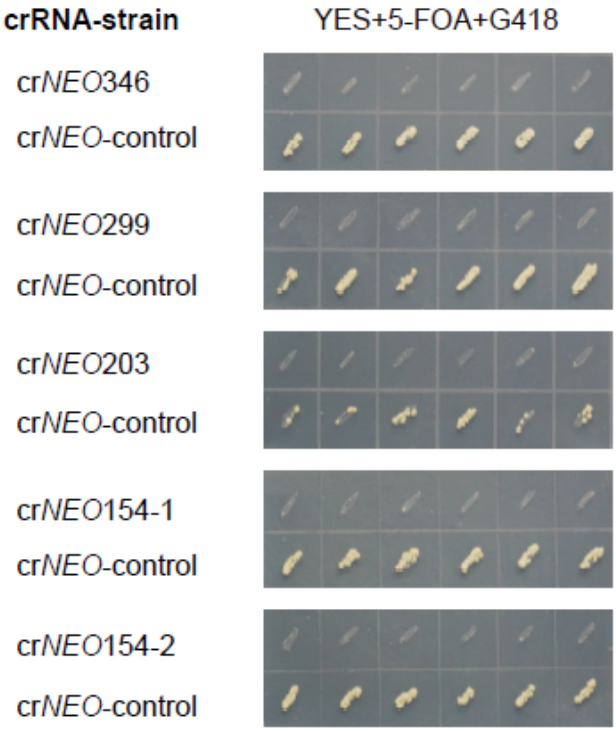


Figure S8. Prolonged crRNA targeting eliminating residual Tf1 retrotransposition by Cas12a.

'Transfer-and-patch' assay for detection of Tf1 retrotransposition using plates containing YES media with 5-FOA and G418. The effects of persistent crRNA targeting in their genome-integrated strains were compared to crNEO-control strains side by side.

Figure S9

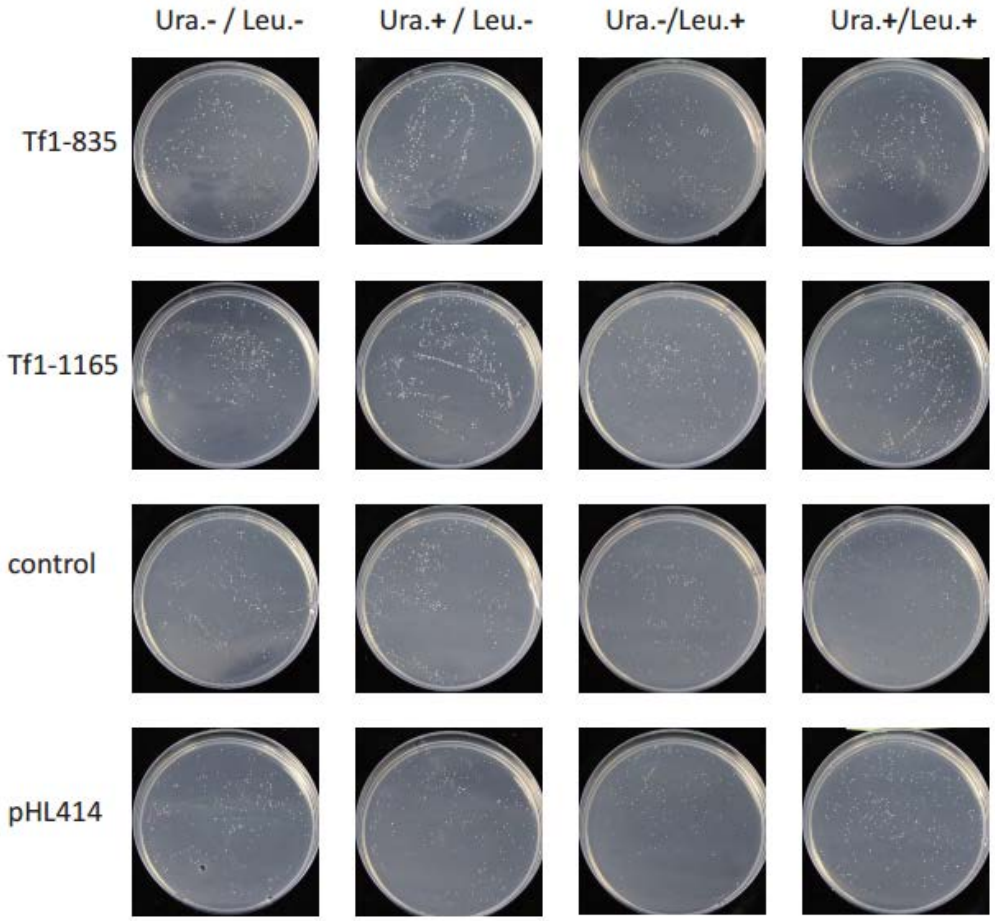


Figure S9. The ending *S. pombe* populations from one batch of liquid cultures (Fig. 5c) were further checked for presence of selection markers for Cas13a gene (Leu+) and Tf1/crRNA-carrying plasmid (Ura+)

100 μL of 1000-fold diluted cells at late log-phase cultures (Fig. 5c) were plated to four different EMM selective plates (uracil-/leucine-, uracil+/leucine-, uracil-/leucine+, and uracil+/leucine+) respectively. The presence of Cas13a gene and Tf1/crRNA-carrying plasmid were validated by colony-formation on selective media plates.

SUPPLEMENTARY TABLES

Supplementary Table S1. List of strains and plasmids used in this study.

Strains and plasmids	Characteristics	Source/Reference
Strains		
E.coli DH5α	*F- eNDA1 glnV44 thi-1 recA1 relA1 gyrA96 deoRnupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17 (rK-mK), λ–*	Takara Biotechnology Co.,Ltd.
S.pombe FY7652	*h- leu1-32 ura4-D18*	National Bio Resource Project
Plasmids		
FnCpf1-OsU6	*E.coli , pBR322 ori ,* Kan^r, FnCpf1, crRNA cassette for expression in rice	Gift from Dr. Jiankang Zhu's Lab[1]
LbCpf1-OsU6	*E.coli , pBR322 ori ,* Kan^r, LbCpf1, crRNA cassette for expression in rice	Gift from Dr. Jiankang Zhu's Lab[1]
pDUAL-HFF1	*E.coli -S.pombe* shutting vector, *ars1 ori*, *ColE1 ori*, *f1 ori* Amp^r , *ura4*	RIKEN BRC (RDB:6179)
pDUAL-HFF1-FnCpf1	*FY7652,* FnCpf1/*nmt1*	This study
pDUAL-HFF1-LbCpf1	*FY7652,* LbCpf1/*nmt1*	This study
pDUAL-HFF1-Cas13a	*FY7652,* LshCas13a/*nmt1*	Our Lab[2]
pHL414	*S. pombe,* Tf1 retrotransposon/*nmt1*	Gift from Professor Henry L. Levin's lab[3]
pBluescript II KS(+)	*E.coli , ColE1 ori, f1 ori,* Amp^r	
pKS-rrk1-FnCpf1-crRNA-backbone	*E.coli,* crRNA backbone plasmid for FnCpf1, Amp^r	This study
pKS-rrk1-LbCpf1-crRNA-backbone	*E.coli,* crRNA backbone plasmid for LbCpf1, Amp^r	This study
pKS-rrk1-LshCas13a-crRNA-backbone	*E.coli,* crRNA backbone plasmid for LshCas13a, Amp^r	This study

Supplementary Table S2. Intermediate plasmids for crRNA constructs.

Intermediate plasmids	Primers	Cloning sites	Original plasmids	Source
pKS-rrk1-(FnCpf1 cr*MEL1*)-NT1	crMEL1-NT1-P5 crMEL1-NT1-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*MEL1*)-NT2	crMEL1-NT2-P5 crMEL1-NT2-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*MEL1*)-T3	crMEL1-T3-P5 crMEL1-T3-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*MEL1*)-T4	crMEL1-T4-P5 crMEL1-T4-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*MEL1*)-T5	crMEL1-T5-P5 crMEL1-T5-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*MEL1*)-control	crMEL1-Control-P5 crMEL1-Control-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-NT1	crMEL1-NT1-P5 crMEL1-NT1-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-NT2	crMEL1-NT2-P5 crMEL1-NT2-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-T3	crMEL1-T3-P5 crMEL1-T3-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-T4	crMEL1-T4-P5 crMEL1-T4-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-T5	crMEL1-T5-P5 crMEL1-T5-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(LbCpf1 cr*MEL1*)-control	crMEL1-Control-P5 crMEL1-Control-P3	*BspQ*I	pKS-rrk1-LbCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*346)	crNEO346-P5 crNEO346-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*299)	crNEO299-P5 crNEO299-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*203)	crNEO203-P5 crNEO203-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*154-1)	crNEO154-1-P5 crNEO154-1-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*154-2)	crNEO154-2--P5 crNEO154-2-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(FnCpf1 cr*NEO*-Control)	crNEO-Control-P5 crNEO-Control-P3	*BspQ*I	pKS-rrk1-FnCpf1-crRNA-backbone	This study
pKS-rrk1-(LshCas13a crRNA)-(Tf1-835)	Tf1-835-gRNA-P5 Tf1-835-gRNA-P3	*BspQ*I	pKS-rrk1-LshCas13a-crRNA-backbone	This study
pKS-rrk1-(LshCas13a crRNA)-(Tf1-1165)	Tf1-1165-gRNA-P5 Tf1-1165-gRNA-P3	*BspQ*I	pKS-rrk1-LshCas13a-crRNA-backbone	This study
pKS-rrk1-(LshCas13a crRNA)-Control	Tf1-Control-gRNA-P5 Tf1-Control-gRNA-P3	*BspQ*I	pKS-rrk1-LshCas13a-crRNA-backbone	This study

Supplementary Table S3. crRNA Constructs for targeting the endogenous *MEL1* gene by FnCpf1 and LbCpf1.

Plasmid Name	Genes/Promoters	crRNA primers	Original plasmid	Descriptions	Source
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-NT1	crRNA/*rrk1*	crMEL1-NT1-P5 crMEL1-NT1-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-NT2	crRNA/*rrk1*	crMEL1-NT2-P5 crMEL1-NT2-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-T3	crRNA/*rrk1*	crMEL1-T3-P5 crMEL1-T3-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-T4	crRNA/*rrk1*	crMEL1-T4-P5 crMEL1-T4-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-T5	crRNA/*rrk1*	crMEL1-T5-P5 crMEL1-T5-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Fn*Cpf1 cr*MEL1*)-control	crRNA/*rrk1*	crMEL1-Control-P5 crMEL1-Control-P3	pDUAL-HFF1	for chromosomal expression of FnCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-NT1	crRNA/*rrk1*	crMEL1-NT1-P5 crMEL1-NT1-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-NT2	crRNA/*rrk1*	crMEL1-NT2-P5 crMEL1-NT2-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-T3	crRNA/*rrk1*	crMEL1-T3-P5 crMEL1-T3-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-T4	crRNA/*rrk1*	crMEL1-T4-P5 crMEL1-T4-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-T5	crRNA/*rrk1*	crMEL1-T5-P5 crMEL1-T5-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-(*Lb*Cpf1 cr*MEL1*)-control	crRNA/*rrk1*	crMEL1-Control-P5 crMEL1-Control-P3	pDUAL-HFF1	for chromosomal expression of LbCpf1 and episomal expression crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-NT1	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-NT1-P5 crMEL1-NT1-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-NT2	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-NT2-P5 crMEL1-NT2-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-T3	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-T3-P5 crMEL1-T3-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-T4	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-T4-P5 crMEL1-T4-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-T5	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-T5-P5 crMEL1-T5-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study
pDUAL-HFF1-*Fn*Cpf1-cr*MEL1*-control	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	crMEL1-Control-P5 crMEL1-Control-P3	pDUAL-FnCpf1-HFF1	for episomal expression of FnCpf1 and crRNA	This study

Supplementary Table S4. crRNA constructs for targeting the Tf1 DNA intermediates (generated via splicing of artificial introns and reverse-transcription) by FnCpf1.

Plasmid Name	Promoter	Original plasmid	Descriptions	Source
pHL414-Tf1-neo-intron346	Tf1/*nmt1*	pHL414	Tf1 retrotransposition reporters plasmid, artificial intron is 346bp from ATG of the NEO gene	This study
pHL414-Tf1-neo-intron299	Tf1/*nmt1*	pHL414	Tf1 retrotransposition reporters plasmid, artificial intron is 299bp from ATG of the NEO gene	This study
pHL414-Tf1-neo-intron203	Tf1/*nmt1*	pHL414	Tf1 retrotransposition reporters plasmid, artificial intron is 203bp from ATG of the NEO gene	This study
pHL414-Tf1-neo-intron154	Tf1/*nmt1*	pHL414	Tf1 retrotransposition reporters plasmid, artificial intron is 154bp from ATG of the NEO gene	This study
pHL414-Tf1-neo-intron346-(*Fn*Cpf1 crNEO346)	Tf1/*nmt1* crRNA/*rrk1*	pHL414-Tf1-neo -intron346	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron299-(*Fn*Cpf1 crNEO299)	Tf1/*nmt1* crRNA/*rrk1*	pHL414-Tf1-neo -intron299	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron203-(*Fn*Cpf1 crNEO203)	Tf1/*nmt1* crRNA/*rrk1*	pHL414-Tf1-neo -intron203	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron154-(*Fn*Cpf1 crNEO154-1)	Tf1/*nmt1* crRNA/*rrk1*	pHL414-Tf1-neo -intron154	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron154-(*Fn*Cpf1 crNEO154-2)	Tf1/*nmt1* crRNA/*rrk1*	pHL414-Tf1-neo -intron154	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron346-(FnCpf1 crNEO-Control)	Tf1/nmt1 crRNA/rrk1	pHL414-Tf1-neo -intron346	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron299-(FnCpf1 crNEO-Control)	Tf1/nmt1 crRNA/rrk1	pHL414-Tf1-neo -intron299	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron203-(FnCpf1 crNEO-Control)	Tf1/nmt1 crRNA/rrk1	pHL414-Tf1-neo -intron203	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pHL414-Tf1-neo-intron154-(FnCpf1 crNEO-Control)	Tf1/nmt1 crRNA/rrk1	pHL414-Tf1-neo -intron154	for chromosomal expression of FnCpf1, episomal expression crRNA and Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*346	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*299	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*203	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*154-1	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*154-2	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study
pDUAL-HFF1-*Fn*Cpf1-cr*NEO*-control	*Fn*Cpf1 / *nmt1* crRNA/*rrk1*	pDUAL-FnCpf1-HFF1	for chromosomal expression of FnCpf1 and crRNA; episomal expression Tf1 retrotransposition reporter	This study

Supplementary Table S5. crRNA constructs for targeting the Tf1 RNA intermediates by LshCas13a.

Plasmid Name	**Promoter**	**Original plasmid**	**target sequence (5' to 3')**	**Descriptions**	**Source**
pHL414-Tf1-(LshCas13a crRNA)-(Tf1-835)	Tf1/*nmt1* crRNA/*rrk1*	pHL414	ATCCAACTAGGTTTAC CATTCTTCTTAA	for chromosomal expression of LshCas13a and episomal expression crRNA and Tf1	This study
pHL414-Tf1-(LshCas13a crRNA)-(Tf1-1165)	Tf1/*nmt1* crRNA/*rrk1*	pHL414	TGTCGTTCTCCTTTAAA AACTTATTGTT	for chromosomal expression of LshCas13a and episomal expression crRNA and Tf1	This study
pHL414-Tf1-(LshCas13a crRNA)-Control	Tf1/*nmt1* crRNA/*rrk1*	pHL414	AATGCCTGGCTTGTCG ACGCATAGTCTG	for chromosomal expression of LshCas13a and episomal expression crRNA and Tf1	This study

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3 Sangesland, M., Atwood-Moore, A., Rai, S. K. & Levin, H. L. Qualitative and quantitative assays of transposition and homologous recombination of the retrotransposon Tf1 in Schizosaccharomyces pombe. *Methods Mol. Biol.* **1400**, 117-130 (2016).

Supplementary Data S1

Primers Name	Sequences (5'-3')	Purpose
Construction of vectors and *S. pombe* strains for FnCpf1 and LbCpf1 expression		
pHSN-Fn-NdeI-F	GCATCACCACCATCAT CATATG gactataaggaccacg	Amplification of FnCpf1 and LbCpf1
pHSN-Fn-NcoI-R	CATCGTCGTCCTTGTAGT CCATGG ttacttttttcttttttgcctg	Amplification of FnCpf1 and LbCpf1
ADHterm-F	CTCTTATTGACCACACCTCTACC	Confirmation of correct integration FnCpf1 and LbCpf1
leu1-R	GGTCATAAAGTTGAACGGATGTCG	Confirmation of correct integration FnCpf1 and LbCpf1
Construction of Tf1 retrotransposition reporters with artificial introns		
neo-intron299	gaatgctgttttcccggggatcgGTAGGTGCTATTTTACTAGTCTAAGCTAA TCAATAGcagtggtgagtaaccatgcatcatcaggagtacggataaaatgcttgatggtcggaa gaggcataaattccgtcagccagtttagtctgaccatctcatctgtaacatcattggcaacgctacctttg ccatgtttcagaaacaactctggcgcatcgggcttcccatacaatcgatagattgtcgcacct	To generate the NEO fragments with artificial intron
neo-intron203	gaatgctgttttcccggggatcgcagtggtgagtaaccatgcatcatcaggagtacggataaaatgctt gatggtcggaagaggcataaattccgtcagccagtttagtctgaccatctGTAGGTGCTATT TTACTAGTCTAAGCTAATCAATAGcatctgtaacatcattggcaacgctacctttg ccatgtttcagaaacaactctggcgcatcgggcttcccatacaatcgatagattgtcgcacct	To generate the NEO fragments with artificial intron
neo-intron154	gaatgctgttttcccggggatcgcagtggtgagtaaccatgcatcatcaggagtacggataaaatgctt gatggtcggaagaggcataaattccgtcagccagtttagtctgaccatctcatctgtaacatcattggca acgctacctttgccatgtttcagaaacaaGTAGGTGCTATTTTACTAGTCTAAG CTAATCAATAGctctggcgcatcgggcttcccatacaatcgatagattgtcgcacct	To generate the NEO fragments with artificial intron
neo-intron346-P5	tcaacaatattttcacctgaGTAGGTGCTATTTTACTAGTCTAAGCTAATC AATAGatcaggatattcttctaatac	To generate the NEO fragments with artificial intron

neo-intron346-P3	gtattagaagaatatcctgatCTATTGATTAGCTTAGACTAGTAAAATAGC ACCTACtcaggtgaaaatattgttga	To generate the NEO fragments with artificial intron
Neo-TYCZ-P5	catcaacaatattttcacctgaatcaggatattcttctaatacctggaatgctgttttcccggggat	To generate the NEO fragments with artificial intron
Neo-TYCZ-P3	atgggtataaatgggctcgcgataatgtcgggcaatcaggtgcgacaatctatcgattg	To generate the NEO fragments with artificial intron

Intermediate plasmids for crRNA constructs

crMEL1-NT1-P5	gat CAGATTGGGGAGTAGATTACTTAAA	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-NT1-P3	gcc TTTAAGTAATCTACTCCCCAATCTG	
crMEL1-NT2-P5	gat CAATAATGATTGGGTAGTGGCTGTT	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-NT2-P3	gcc AACAGCCACTACCCAATCATTATTG	
crMEL1-T3-P5	gat GTCCCAAACCATTATAGCTACCATG	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-T3-P3	gcc CATGGTAGCTATAATGGTTTGGGAC	
crMEL1-T4-P5	gat CTTCCAATCTACCAGTGGTTGCATT	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-T4-P3	gcc AATGCAACCACTGGTAGATTGGAAG	
crMEL1-T5-P5	gat AGTAATCTACTCCCCAATCTGCAAA	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-T5-P3	gcc TTTGCAGATTGGGGAGTAGATTACT	
crMEL1-Control-P5	gat AACAGCGCCTTAAAAGAACTAGAAA	the oligonucleotide pairs designed for spacer of each crRNA
crMEL1-Control-P3	gcc TTTCTAGTTCTTTTAAGGCGCTGTT	
crNEO346-P5	GAT acctgaatcaggatattcttctaat	the oligonucleotide pairs designed for spacer of each crRNA
crNEO346-P3	GCC attagaagaatatcctgattcaggt	
crNEO299-P5	GAT ccggggatcgcagtggtgagtaacc	the oligonucleotide pairs designed for spacer of each crRNA
crNEO299-P3	GCC ggttactcaccactgcgatccccgg	
crNEO203-P5	GAT gtctgaccatctcatctgtaacatc	the oligonucleotide pairs designed for spacer of each crRNA
crNEO203-P3	GCC gatgttacagatgagatggtcagac	

crNEO154-1-P5	GAT ccatgtttcagaaacaactctggcg	the oligonucleotide pairs designed for spacer of each crRNA
crNEO154-1-P3	GCC cgccagagttgtttctgaaacatgg	
crNEO154-2-P5	GAT agaaacaactctggcgcatcgggct	the oligonucleotide pairs designed for spacer of each crRNA
crNEO154-2-P3	GCC agcccgatgcgccagagttgtttct	
crNEO-Control-P5	gat AACAGCGCCTTAAAAGAACTAGAAA	the oligonucleotide pairs designed for spacer of each crRNA
crNEO-Control-P3	gcc TTTCTAGTTCTTTTAAGGCGCTGTT	
Tf1-835-gRNA-P5	aac Atccaactaggtttaccattcttcttaa	the oligonucleotide pairs designed for spacer of each crRNA
Tf1-835-gRNA-P3	gcc ttaagaagaatggtaaacctagttggat	
Tf1-1165-gRNA-P5	aac Tgtcgttctcctttaaaaacttattgtt	the oligonucleotide pairs designed for spacer of each crRNA
Tf1-1165-gRNA-P3	gcc aacaataagtttttaaaggagaacgaca	
Tf1-Control-gRNA-P5	acc CAGACTATGCGTCGACAAGCCAGGCATT	the oligonucleotide pairs designed for spacer of each crRNA
Tf1-Control-gRNA-P3	gcc AATGCCTGGCTTGTCGACGCATAGTCTG	

Construction of crRNA vectors to direct targeting by FnCpf1, LbCpf1, and LshCas13a

pDUAL-PspXI-T7	CCTCCAATCTTGTGTTCTTCAAA TAATACGACTCACTATAGG	Amplification of crRNA array cassettes inserted into linearized plasmids pDUAL-HFF1
pDUAL-SpeI-T3	CGCTAGGGATAACAGGGTAATAT AATTAACCCTCACTAAAGG	Amplification of crRNA array cassettes inserted into linearized plasmids pDUAL-HFF1
pHL414-NheI-T7	TGCAGCCCGGGGGATCCCAGCTGTAATACGACTCACTATAG	Amplification of crRNA array cassettes inserted into linearized plasmids of Tf1 retrotransposition reporters
pHL414-NheI-T3	ATCGCCAGTCACTATGGCGTGCTctagaAATTAACCCTCACTAAAG	Amplification of crRNA array cassettes inserted into linearized plasmids of Tf1 retrotransposition reporters

pDUAL-SpeI-SK primer	ATCGACGATAAAAGAATCATCTTctagaactagtggatc	Amplification of crRNA array cassettes inserted into linearized plasmid pDUAL-HFF1-FnCpf1
pDUAL-SpeI-T3	CGCTAGGGATAACAGGGTAATATAATTAACCCTCACTAAAGG	Amplification of crRNA array cassettesinserted into linearized plasmid pDUAL-HFF1-FnCpf1
NheI-TYB-P5	GGGGGATCCCAGCTGGCTAGCAATTAACCCTCACTAAAGG	Amplification of crRNA array cassettes inserted into linearized plasmid pHL414
NheI-TYB-P3	TCACTATGGCGTGCTGCTAGCTAATACGACTCACTATAGG	Amplification of crRNA array cassettes inserted into linearized plasmid pHL414
MEL1-repair fragment		
mel1-left-P5-new	AGGTTAAAACTCCAGAACATTAC	Amplification of repair fragment for *MEL1* deletion
mel1-left-P3-new	TCATGTGCTAGGTCGATTCTGG GAACAAGAAGTCAATATCGCTTAC	Amplification of repair fragment for *MEL1* deletion
mel1-right-P5-new	GTAAGCGATATTGACTTCTTGTTC CCAGAATCGACCTAGCACATGA	Amplification of repair fragment for *MEL1* deletion
mel1-right-P3-new	GCTATAGTAGAACGAGCCCCGA	Amplification of repair fragment for *MEL1* deletion
MEL1-P5	AGGTTAAAACTCCAGAACATTAC	Confirmation *MEL1* deletion
MEL1-P3	GCTATAGTAGAACGAGCCCCGA	Confirmation *MEL1* deletion

Supplementary Data S2

Interference with Tf1 retrotransposition by CRISPR-Cas12a leaving marginal transposition activities

crRNA-plasmid	# of colonies	YE+Ura.+5-FOA --10^5 cell / mL	YE+Ura.+5-FOA+G418 --10^6 cell / mL	Transposition frequency(%)	Normalized - Transposition frequency(%)
cr*NEO* 346	crNEO346-①	1500	12	0.08	
	crNEO346-②	2500	14	0.06	
	crNEO346-③	2795	19	0.07	
	mean (crNEO346)			0.07	0.1
cr*NEO* control	crNEO-control-1-①	2000	74	0.37	
	crNEO-control-1-②	2000	86	0.43	
	crNEO-control-1-③	2000	160	0.80	
	crNEO-control-2-①	2600	259	1.00	
	crNEO-control-2-②	2200	228	1.04	
	crNEO-control-2-③	2300	210	0.91	
	mean (crNEO-control)			0.76	1.0

Prolonged crRNA targeting eliminating residual Tf1 retrotransposition by CRISPR-Cas12a

crRNA-strain	# of colonies	YE+Ura.+5-FOA --10^5 cell / mL	YE+Ura.+5-FOA+G418 --10^7 cell / mL	Transposition frequency(%)	Normalized - Transposition frequency(%)
cr*NEO* 346	crNEO 346-①	2200	1	0.000	
	crNEO 346-②	2000	1	0.001	
	crNEO 346-③	2500	1	0.000	
	mean (crNEO 346)			0.000	0.00
cr*NEO* control	crNEO control-①	2500	1220	0.488	
	crNEO control-②	2500	1214	0.486	
	crNEO control-③	2500	1257	0.503	
	mean (crNEO control)			0.492	1.00

Interfering with Tf1 retrotransposition by CRISPR-Cas13a via targeting its RNA intermediates

crRNA-plasmid	# of colonies	YE+Ura.+5-FOA+G418 --10^6 cell / mL	YE+Ura.+5-FOA --10^5cell / mL	Transposition frequency(%)	Normalized - Transposition frequency(%)
Tf1-835	Tf1-835-①	820	2100	3.905	
	Tf1-835-②	800	2000	4.000	
	Tf1-835-③	870	2000	4.350	
	mean (Tf1-835-1)			4.085	0.84
Tf1-1165	Tf1-1165-①	340	1700	2.000	
	Tf1-1165-②	400	2150	1.860	
	Tf1-1165-③	400	2020	1.980	
	mean(Tf1-1165-3)			1.947	0.40
Tf1-control	Tf1-control-①	970	1900	5.105	
	Tf1-control-②	1020	2000	5.100	
	Tf1-control-③	910	2100	4.333	
	mean (Tf1-control)			4.846	1.00