Supplementary Information

Genome targeting by hybrid Flp-TAL recombinases

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Supplementary Note 1. Target specificity of Flp-TAL recombinases, the Flp module of which can recombine multiple *FRT*-like sequences.

Target specificity of the hybrid Flp-TAL recombinases is the product of target specificity of the TAL and the Flp modules. For the sake of this analysis, we consider target specificity of the TAL modules a fixed value and estimate the overall specificity of Flp-TAL that contain the Flp module with broad target specificity by assessing the probability to find an *FRT*-like sequence between two binding sequences for the TAL modules (see Figs. 1 and 2).

Target specificity of the Flp module that is capable of recombining several *FRT*-like sequences can be estimated based on the sequence characteristics of the *FRT*-like sequences that differ them from random DNA sequences. In mammalian genomes, these sequence characteristics translate into one *FRT*-like sequence per about 5,000 base pairs (Shultz et al., 2011). As such, the probability to find an *FRT*-like sequence between the two sequences for the TAL modules is ~1/5,000. Consequently, the target specificity of Flp-TAL should be about three orders of magnitude higher than that of the TAL modules.

Additionally, we have to consider the important property of the Flp/*FRT* system that depends on the spacer sequence of the recombination target to function: only the targets with the same spacers will efficiently recombine with each other while the targets with different spacers will not. Since *FRT* has an 8-bp spacer, the probability to find a spacer sequence of this length is $1/4^8$ (or 1/65,536). For the high-scoring *FRT*-like sequences this probability is higher since the first and the last base pairs of the spacer in these sequences are invariant: T/A and A/T, respectively, and the G/C content of the spacer is set to be equal or lower than 50% (Shultz et al., 2011). Collectively, these spacer features increase the probability to find two *FRT*-like sequences with the same spacer to $1/4^6/2$ (or ~1/2,000).

Taken together, the theoretical probability to find an *FRT*-like sequence with a unique spacer between two TAL binding sequences is ~$1/10^7$ (~$1/(5x10^3)$ x ~$1/(2x10^3)$). Such low probability should ensure that the TAL-guided Flp variant with broad target specificity will recombine just the *FRT*-like sequence of interest. This, of course, can only be realized if the Flp module is not sufficiently active to recombine the *FRT*-like sequences on its own, without being stabilized on the target by the TAL module.

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... ATCCTACATAAATAGACGCATA GGTACC
GGATCAGGCGGAAGCGGAGGCTCAGGCGGAAGCGGCACATCA
GTGGATCTACGCACGCTCGGCTACAGTC ...
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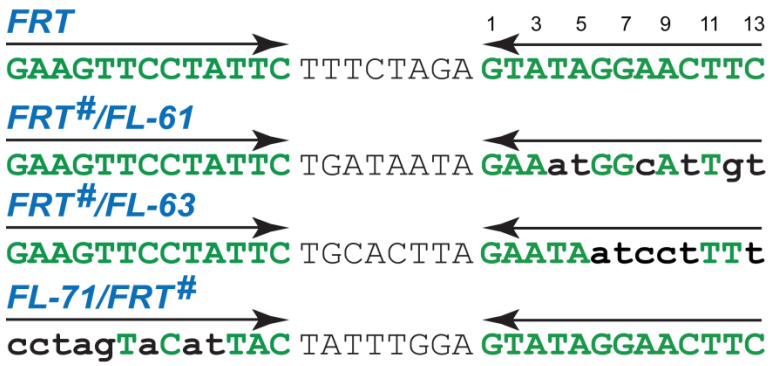
Supplementary Figure 1. Sequence of the fusion between the Flp and the TAL genes.



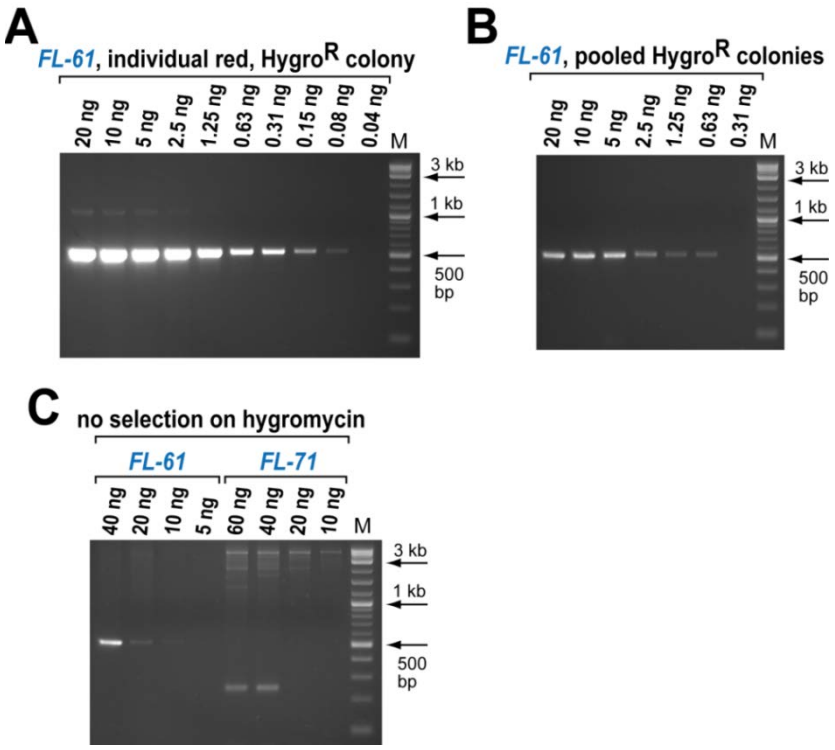
Supplementary Figure 2. Hybrid *FRT*#/*FL-61*, *FRT*#/*FL-63*, and *FL-71/FRT*# sequences. *FRT* is shown above the sequences for comparison.

Colony color	Recombinases			
	FV61-TAL	**FV63-TAL**	**FV71-TAL**	**Control**
Red	80±33	125±36	94±29	52±22
Green	885±201	493±114	295±54	1240±146
Red/Green	381±117	750±129	334±39	1100±186
Colorless	386±91	325±48	154±48	149±43

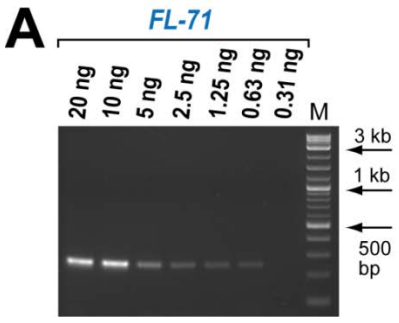
Supplementary Table 1. Average number of colony types in the integration experiments performed with the FV61-TAL, FV63-TAL, and FV71-TAL recombinases. Control experiments were performed with the recombinase R1-6 (Voziyanova et al., 2015), which cannot recognize the *FRT*-like sequences in pTarget. The integration experiments were performed in three biological replicates with the same batch of the vectors.

	Recombinases		
	FV61-TAL	**FV63-TAL**	**FV71-TAL**
Integration-positive colonies	9 (81) 8 (67)	7 (73) 9 (76)	5 (80) 3 (77)

Supplementary Table 2. Fraction of the integration-positive colonies obtained in the FV61-TAL, FV63-TAL, and FV71-TAL experiments. The number of the integration-positive colonies are shown and the total number of the hygromycin-resistant colonies of the respective color analyzed (red in the FV61-TAL and FV63-TAL experiments and green in the FV71-TAL experiments) are given in parenthesis.



Supplementary Figure 3. PCR-based assessment of the Flp-TAL integration efficiency. PCR analyses of (**A**) genomic DNA isolated from individual hygromycin-resistance, integration-positive colony, (**B**) from the pooled hygromycin-resistance colonies that were generated in the *FL-61* targeting experiments (see Fig. 4), and (**C**) from pooled cells that were co-transfected with pTarget and FV61-TAL and FV71-TAL, respectively, and then expanded in the growth medium without hygromycin. The PCR analyses were performed at the right junction between the integrated pTarget and genome DNA (Fig. 4). M, 2-log DNA ladder (NEB).



B

Exp 1	Exp 2	Exp 3
0.63 ng	0.63 ng	0.31 ng

Supplementary Figure 4. PCR-based assessment of the deletion activity of FV71-TAL. (**A**) PCR analysis of genomic DNA isolated from the *FL-61* integration-positive cells that were transfected with FV71-TAL (see Fig. 4a). (**B**) The experiments were performed in three biological replicates; the results of these experiments are indicated. The PCR analyses were

performed at the right junction between the integrated pTarget and genome DNA (see Fig. 6b). M, 2-log DNA ladder (NEB).

Supplementary Table 3. Oligonucleotide pairs that were used to PCR amplify control DNA fragments in the targeting experiments in human cells

Region	Oligonucleotide sequence
Left Junction	
FL-61	GTATCCAAGACTGTATTTCTGATTTTATCGTACC (anneals at ~90 bp upstream of *FL-61*) CCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTG (anneals in the middle of the EGFP gene in pTarget)
FL-63	CTGATAGGCACTGACTCTCTGTCCCTTGGGCTG (anneals at ~550 bp upstream of *FL-63*) CCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTG (anneals in the middle of the EGFP gene in pTarget)
FL-71	GGTTAAGTTCATGTCATAGGAAGGGGATAAGTAACAGGG (anneals at ~280 bp upstream of *FL-71*) GGTGGCGAGGCGCACCGTGGGCTTGTACTCGG (anneals at the beginning of the puro-2A-DsRed gene in pTarget)
Right Junction	
FL-61	GGACTTTCCAAAATGTCGTAACAACTCCGC (anneals at the 3'-end of the CMV promoter in pTarget) AAAGATGGATGATGTGCCTGAGATTCTGATCACAAGG (anneals at ~270 bp downstream from *FL-61*)
FL-63	CATCGGCATAGTATATCGGCATAGTATAATACGAC (anneals at the 5'-end of the recombination target cassette I in pTarget) CTTAGACAAAACTGATCCCCAGGTTATTCCCATCAG (anneals at ~490 bp downstream from *FL-63*)
FL-71	GCAAAACCTTTCGCGGTATGGCATGATAGCGCCC (anneals at the 5'-end of the recombination target cassette II in pTarget) CAATATGTGTACACATATTAAAACATTACACTTTAACCC (anneals at ~115 bp downstream from *FL-71*)

Supplementary Table 4. *FRT*-like sequences in the human genome that were tested for potential off-target integration events. The *FRT*-like sequences shown have the highest level of homology to *FRT* (Shultz et al., 2011) and their spacer sequences, which are highlighted in bold, are identical to that of *FL-61*, see Fig. 2b. The relative orientation of these *FRT*-like sequences (which is specified by the direction of their spacer sequences) is different in the human chromosomes but these sequences are arranged in such a way that the direction of their spacers matches that of *FL-61* (Fig. 2b).

***FRT*-like sequence**	
AACAATCCTATTC**TGATAATA**AAATAAAGCTGAT,	**Chr-01**
TATTATCATATTT**TGATAATA**AAATATGATTTGA,	**Chr-01**
AGTTTTCCAATAT**TGATAATA**GTATAAACAAGCA,	**Chr-01**
GATTGGGCAATTT**TGATAATA**AAATAAGAATTCC,	**Chr-02**
ATGTTTCTGATAC**TGATAATA**GTATAAGTGCCAA,	**Chr-02**
AGTTCGCAAATTC**TGATAATA**GAATTGCTCCTCT,	**Chr-03**
AGAATAATTATTC**TGATAATA**ATATTAGTAATAA,	**Chr-03**
AATTTTCTTATTT**TGATAATA**GTATAATGGTTAT,	**Chr-03**
TAAGTGAATATTT**TGATAATA**AAATTGGAGATCA,	**Chr-04**
GCTTGCCTGATTC**TGATAATA**ATATTCAAGCAAA,	**Chr-04**
AGTTTTCTTATAT**TGATAATA**AAATTGTTAATGT,	**Chr-04**
GTTTCACATATAT**TGATAATA**GTATCACCATATC,	**Chr-06**
TGACTTTTGATAC**TGATAATA**GAATAAGCACATT,	**Chr-06**
ACCAGTATGATTC**TGATAATA**GAATAGGTTGAGT,	**Chr-08**
ACCAGTATCATTT**TGATAATA**AAATCTGACAGAG,	**Chr-11**
TGCAAACAGATAC**TGATAATA**GAATATGAAATAA,	**Chr-11**
CACCAATTGATAC**TGATAATA**AAATATGTTCATT,	**Chr-11**
TTTAGTATAATTT**TGATAATA**AAATAAGAGTAAT,	**Chr-12**
TTTTCCAATATAT**TGATAATA**AAATAGGTTTGCT,	**Chr-12**

GATTTCCTAATTT**TGATAATA**GTATAGTGCTTAT, **Chr-13**

CAGTAAATGATAT**TGATAATA**GTATAAGTACTGT, **Chr-14**

GTCTGTCATATTC**TGATAATA**ATATGATTTAATA, **Chr-14**

AAGACATCAATAC**TGATAATA**GTATATGATGTTA, **Chr-14**

AATATACTCATTT**TGATAATA**AAATATAGTATGC, **Chr-18**

TTGATATTAATAT**TGATAATA**ATATCAGTATGTC, **Chr-21**

AGATAACCAATAT**TGATAATA**ATATAATAATCAT, **Chr-X**

ATCTCTCTCATAT**TGATAATA**ATATATCAATTGG, **Chr-X**

CATCAATTTATAC**TGATAATA**AAATTTGAATTTT, **Chr-X**

TATGGGATCATAC**TGATAATA**GTATAAGGGTTCG, **Chr-Y**

pTarget vector sequence (without recombination targets)

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT
AAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACA
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CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCA
CTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCC**AAGCTT**

Recombination target cassette I

CTTCcctttgaaaaacacgatgataatatggccacaacc**ATG**GTGAGCAAGGGCGAGGAGCTGTTCACCG
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Recombination target cassette II

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CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATC
TTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG
GCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCC
CCGAAAAGTGCCACCTGACGTC

Recombination target cassettes I

FRT#/FL-61

CATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACC
CCTATTCTTGTGTTCACGACTGACATCACCGT GAAGTTCCTATAC **TGATAATA**
GAAat**GG**c**A**t**T**gt CACTT**T**CTTCCCTACTGCAACAGAAGCCCAGCT

FRT#/FL-63

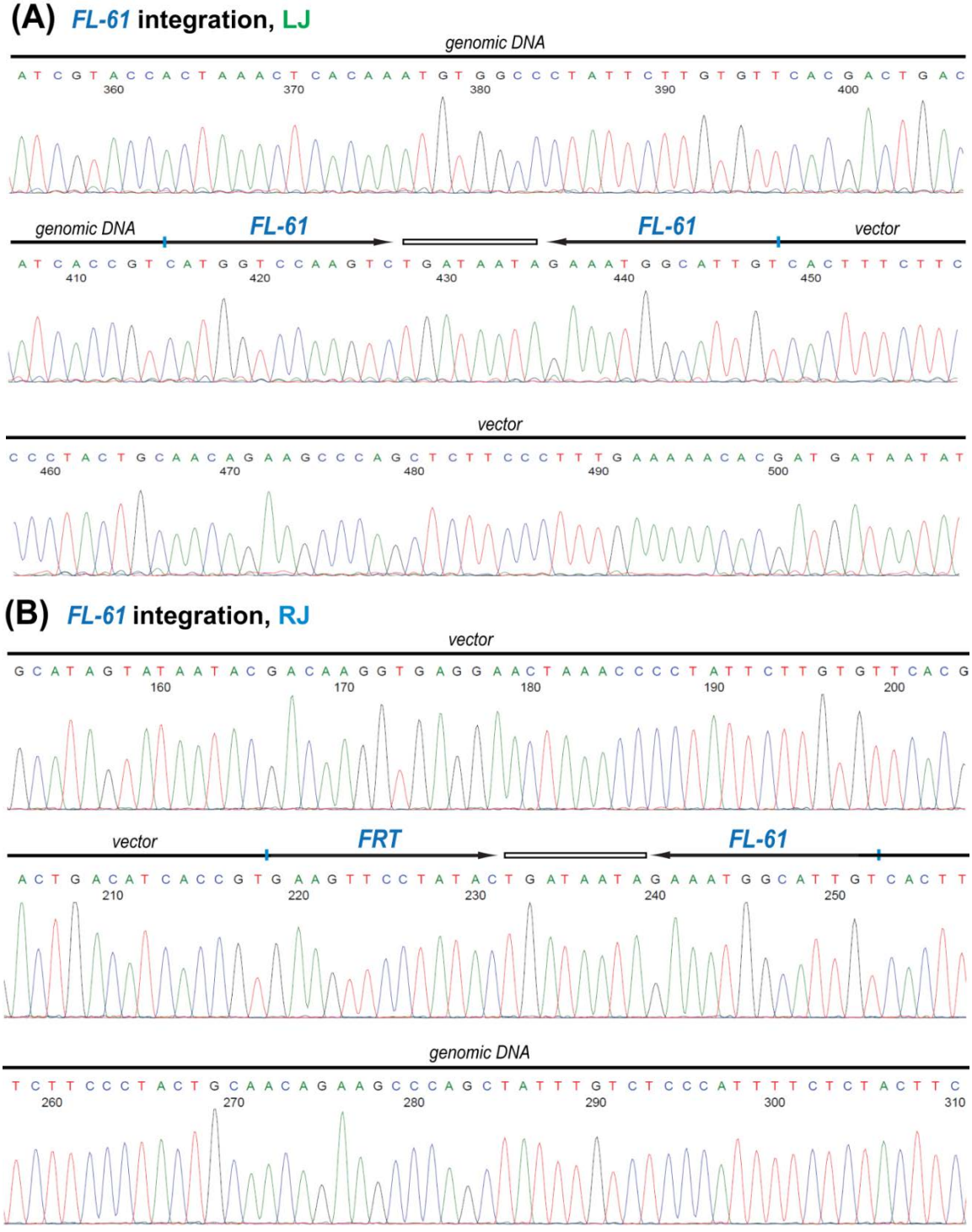
CATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACC
ACAACTCAAATTATGCATACCAGCTCT GAAGTTCCTATAC **TGCACTTA** GAATAATCCTTTT
GTCTC**T**CCACATGGGTATGGGAGAGGC

Recombination target cassette II

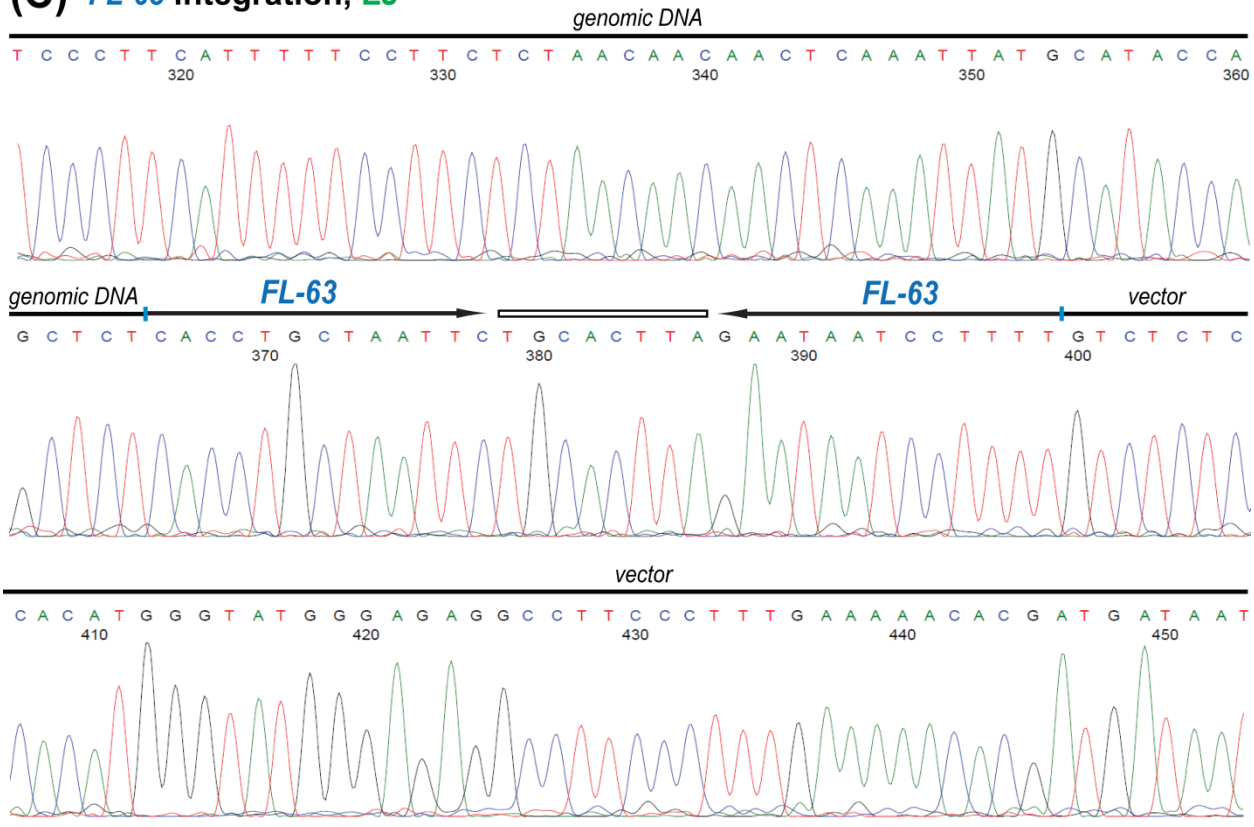
FL-71/FRT#

tggtgcaaaacctttcgcggtatggcatgatagcgcccggaagagagtcaattcagg
GAGATACATTAAGTAACTTAAAAAAAAACTTTACACAGTCTG cctag**T**a**C**at**TAC**
TATTTGGA GTATAGGAACTTC TATT**T**GCATATTCATAATCTCCCTACTTTATTTTCT

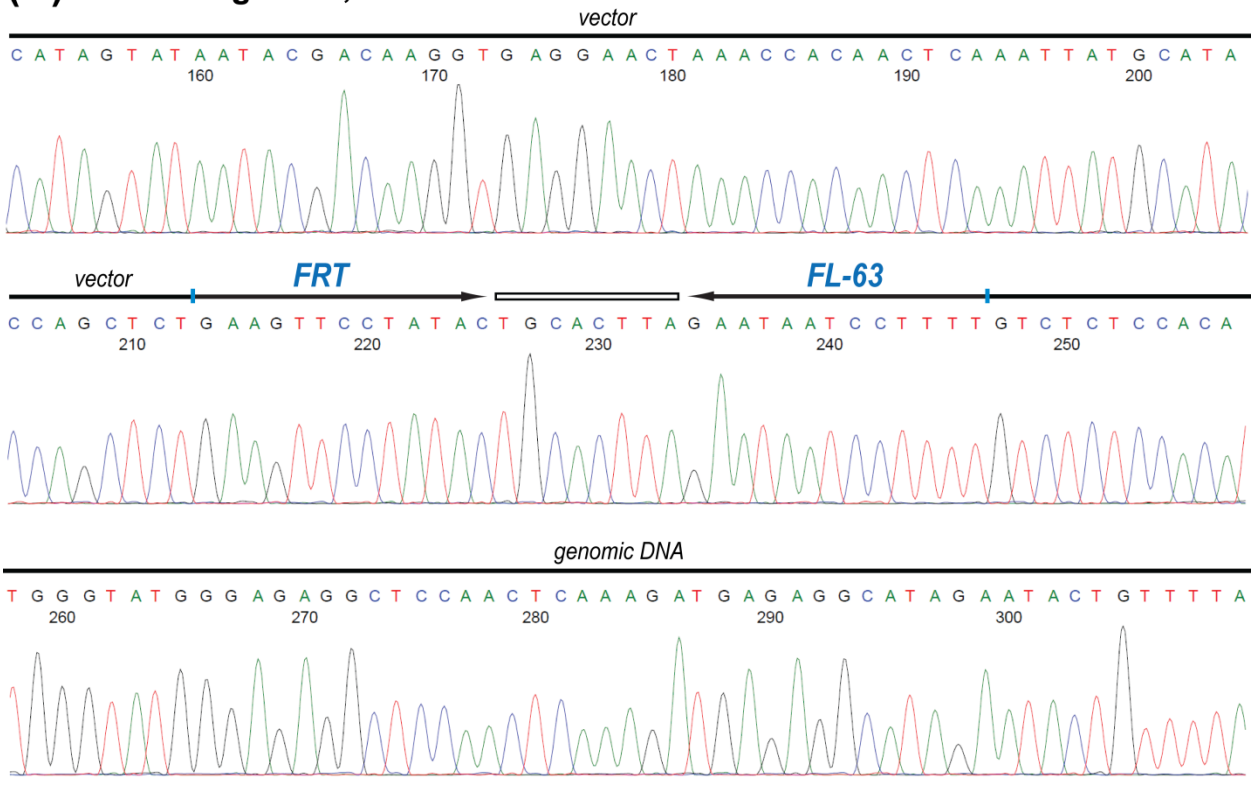
Supplementary Figure 5. Partial sequencing traces of the diagnostic PCR bands shown in Figures 4B and 6B. (**A**) and (**B**), FV61-TAL dependent integration of pTarget into *FL-61;* LJ and RJ, respectively. (**C**) and (**D**), FV63-TAL dependent integration of pTarget into *FL-63;* LJ and RJ, respectively. (**E**) and (**F**), FV71-TAL dependent integration of pTarget into *FL-71;* LJ and RJ, respectively. (**G**) FV71-TAL dependent deletion of pTarget and genomic sequences*;* RJ, respectively.



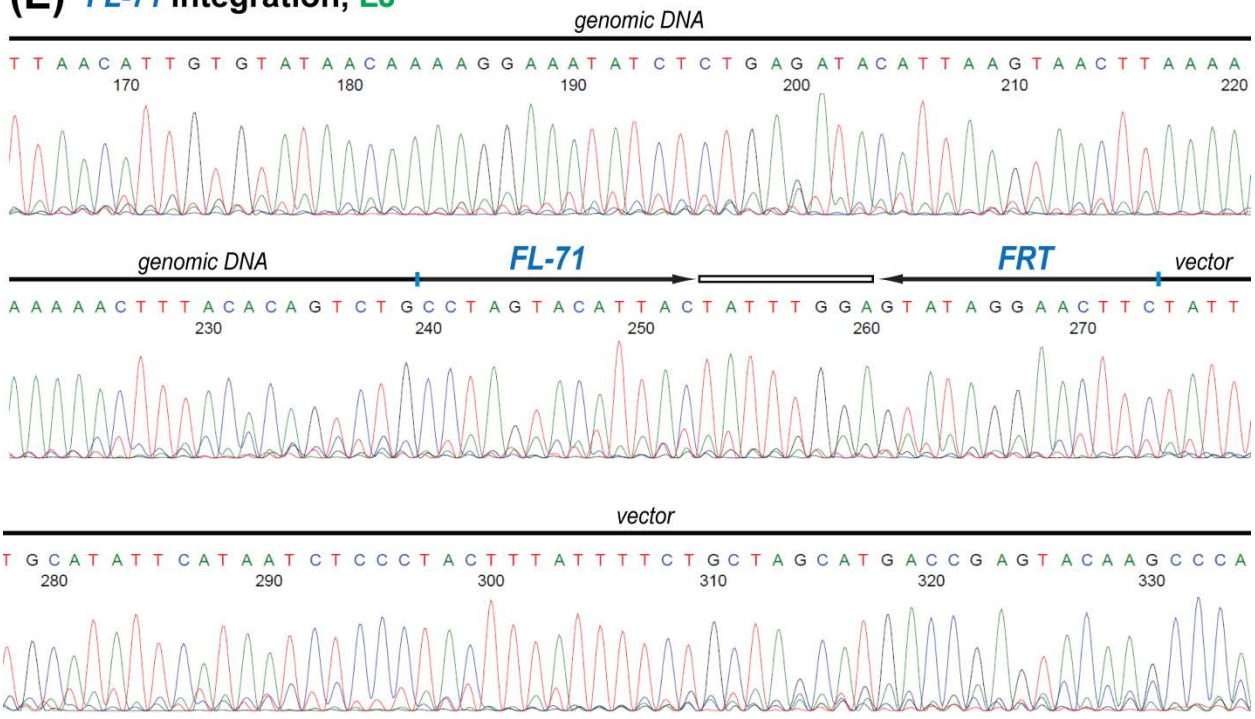
(C) *FL-63* integration, LJ



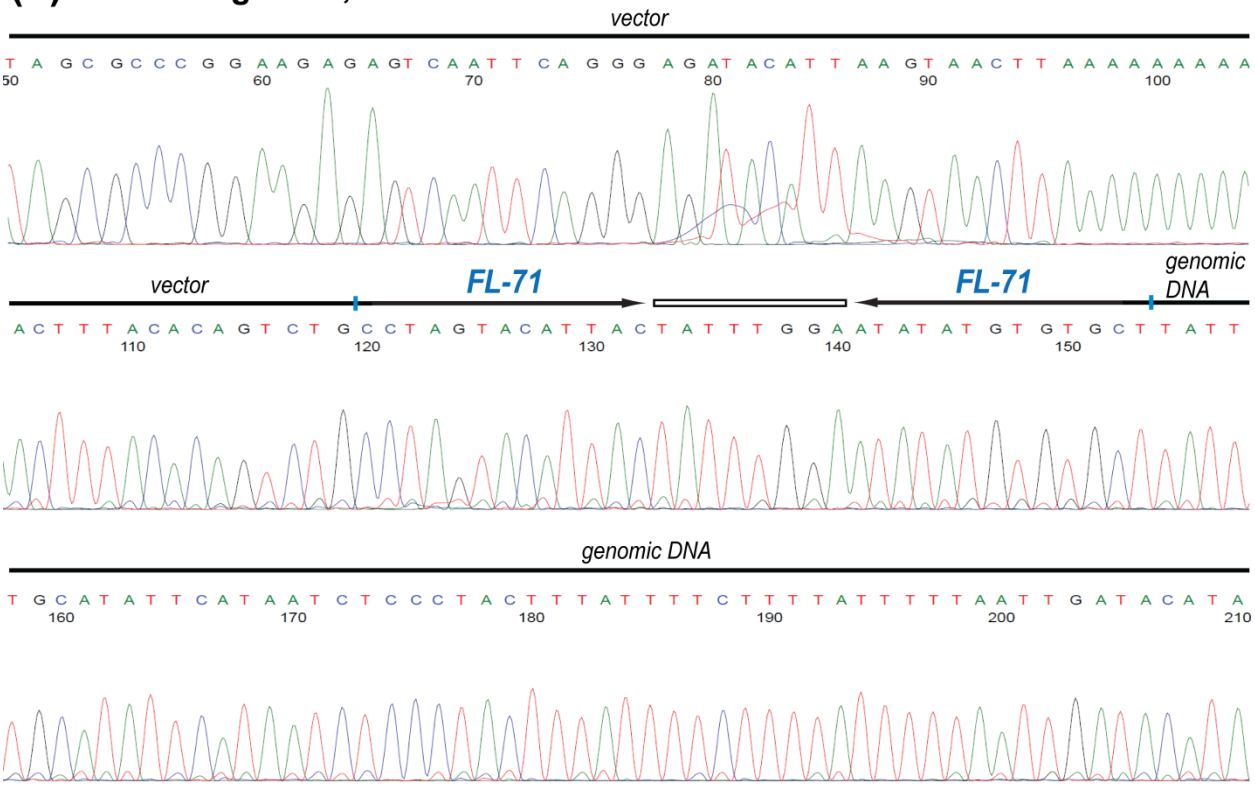
(D) *FL-63* integration, RJ



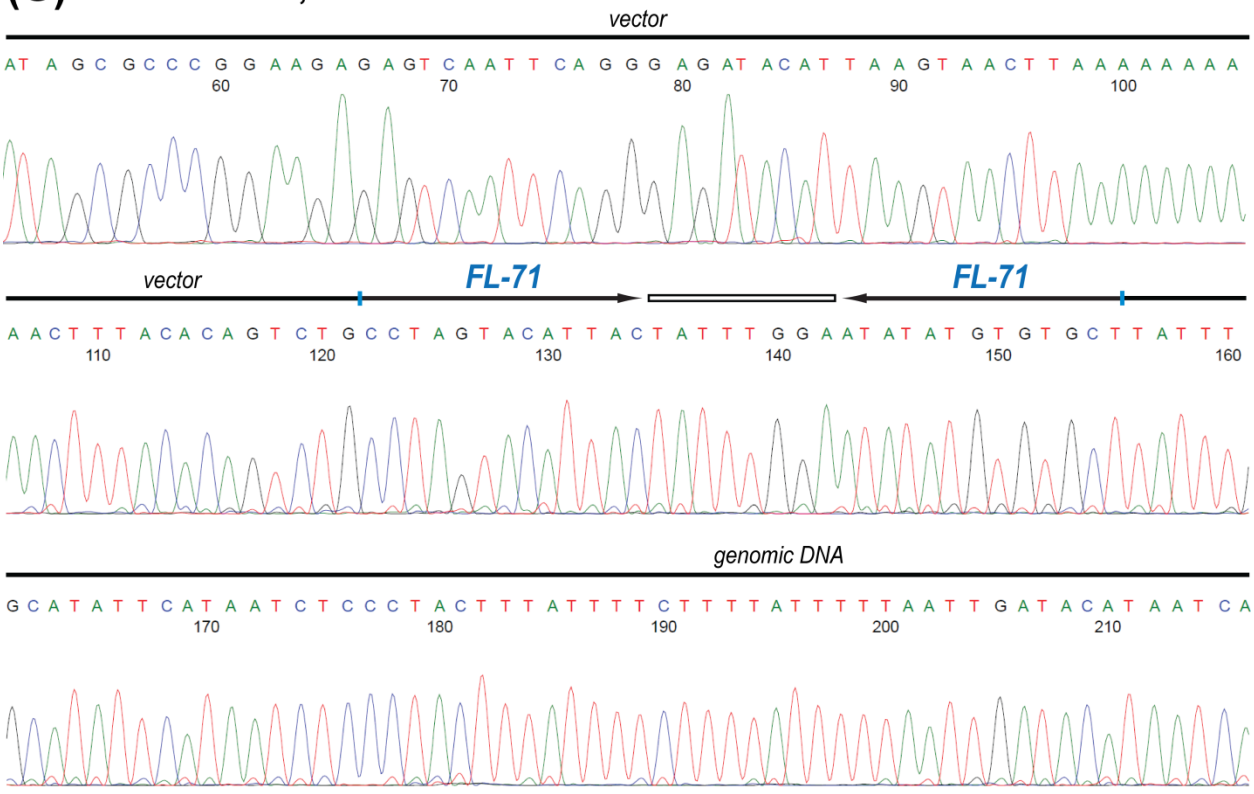
(E) *FL-71* integration, LJ



(F) *FL-71* integration, RJ



(G) *FL-71* deletion, RJ



Supplementary Figure 6. Original images of the gels.

Figure 3E.

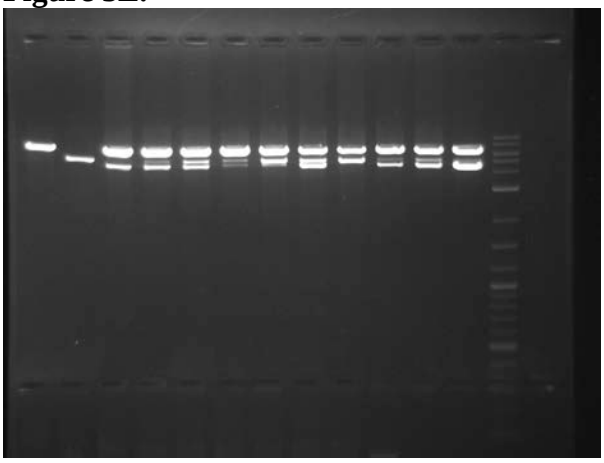


Figure 4B.

LJ **RJ**

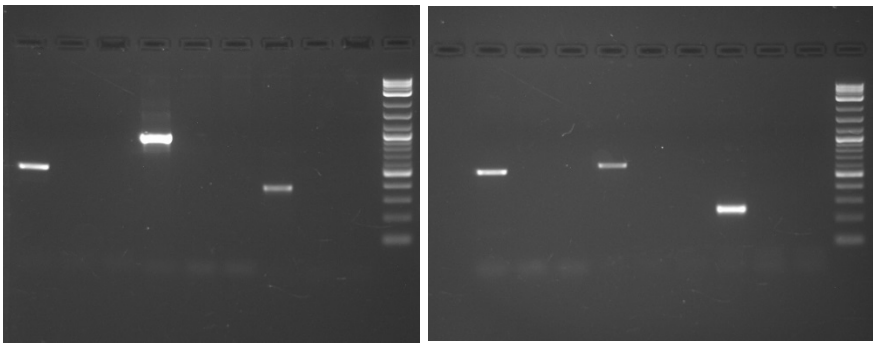


Figure 4D.

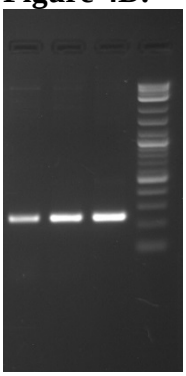


Figure 5A and B.

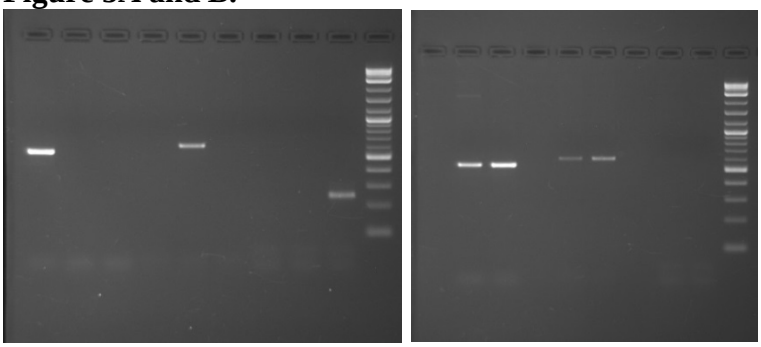
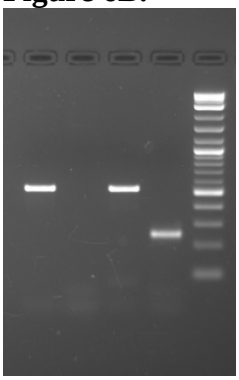
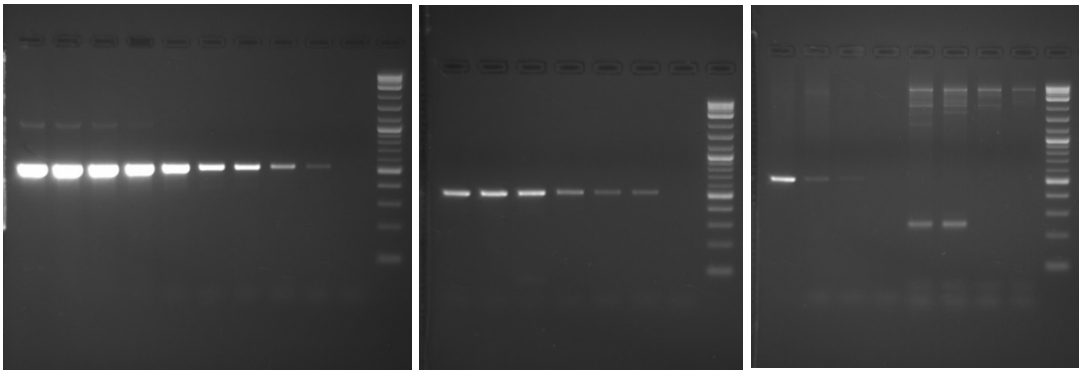


Figure 6B.



Supplementary Figure 3A, B, and C.

A **B** **C**



Supplementary Figure 4A.