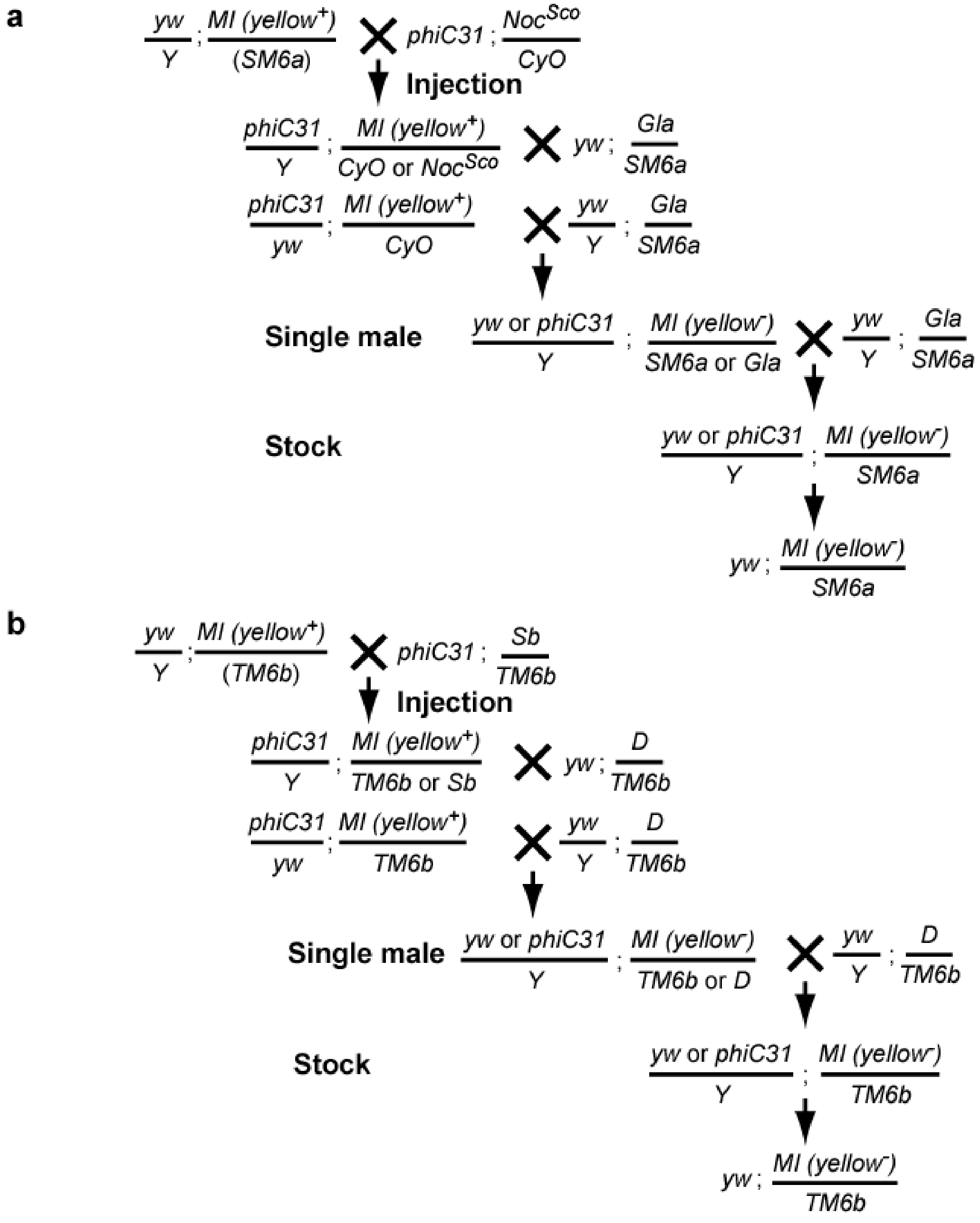
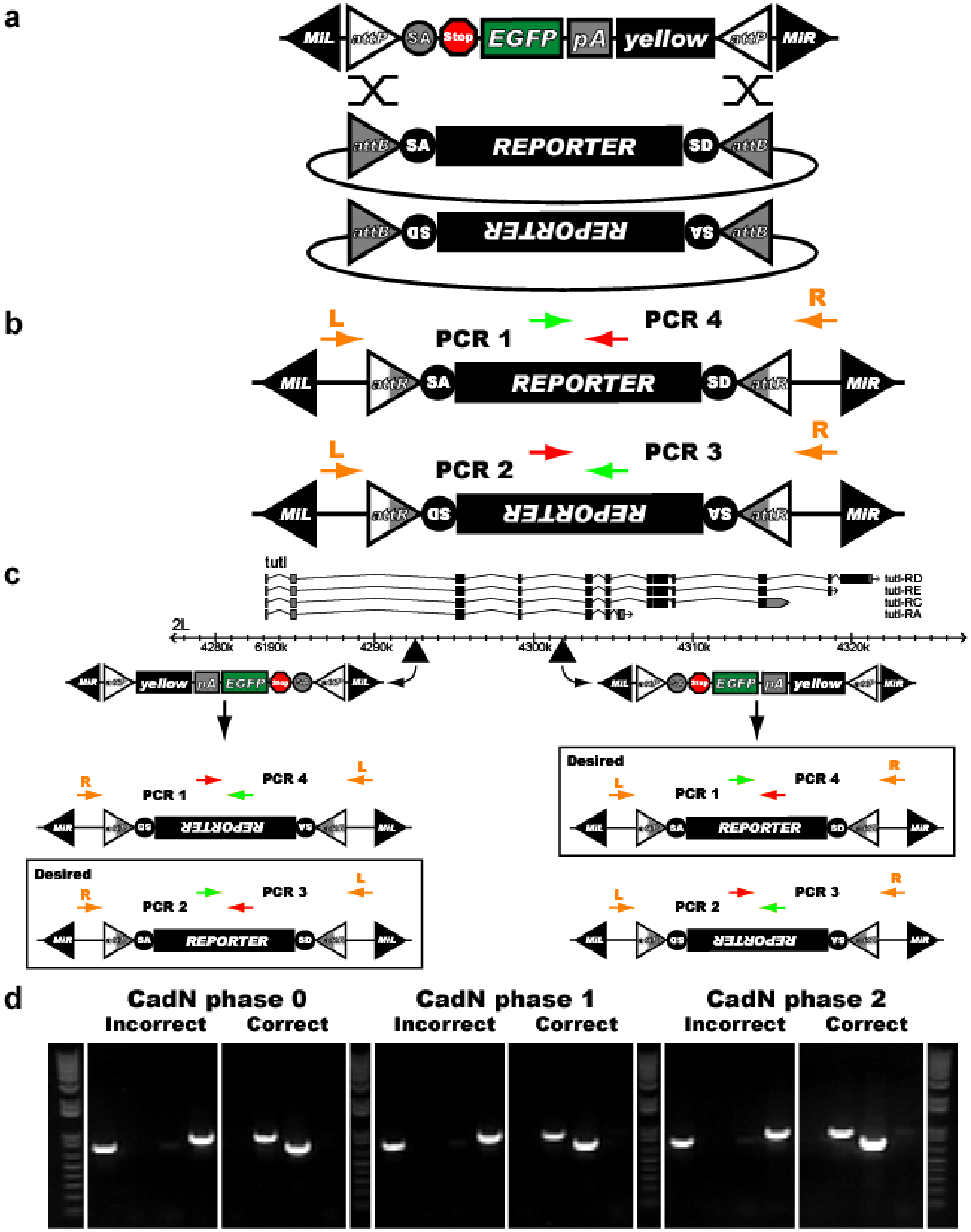
Supplementary Figure 1



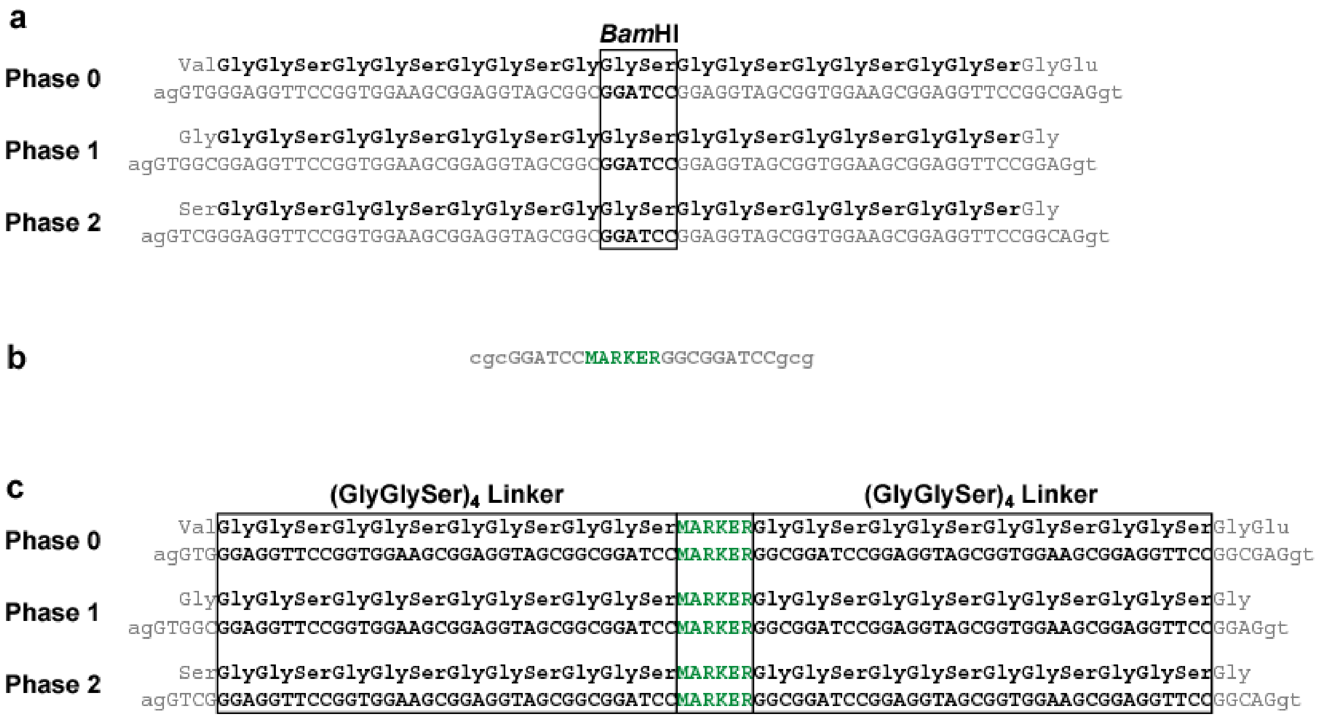
Supplementary Figure 1. Crossing schemes for RMCE of MiMIC insertions on the second and third chromosomes. (**a**) Crosses for chromosome 2. (**b**) Crosses for chromosome 3. The phiC31 chromosome is *yw*.

Supplementary Figure 2



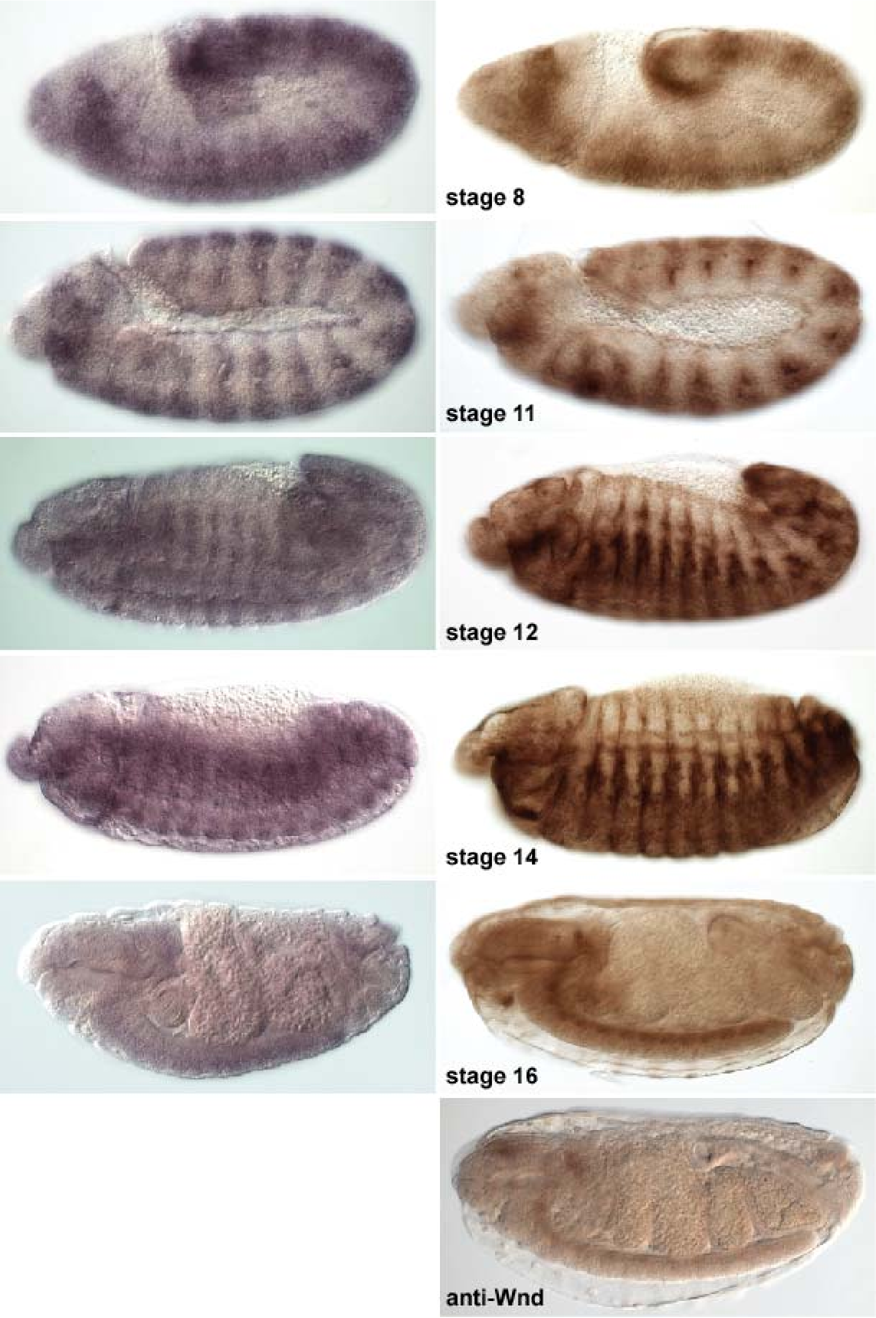
Supplementary Figure 2. Orientation of RMCE events within MiMIC insertions. (**a**) RMCE with inverted *attP* sites flanking the gene trap and marker gene within the MiMIC transposon and inverted *attB* sites flanking the replacement cassette within a plasmid construct can result in the integration of the cassette in either of two orientions, one of which results in the desired allele. (**b**) Four PCR reactions distinguish between integration in the two orientations: a RMCE event is either positive for PCR reactions 1 and 4 or positive for PCR reactions 2 and 3. The desired PCR results depend on the orientation of the MiMIC insertion relative to the gene. (**c**) Illustrations of PCR results for the possible outcomes of RMCE events in two MiMIC insertions in the *tutl* gene. With *tutl* located on the forward strand and a MiMIC insertion on the reverse strand (Left), a “2/3” PCR pattern indicates a productive RMCE event. For a MiMIC insertion on the forward strand (Right), a “1/4” PCR pattern indicates a productive RMCE event. (**d**) PCR results for RMCE events in *CadN* using the 3 different splice phase plasmids containing the EGFP-FlAsH-StrepII-3xFlag tag. Only the correct “2/3” PCR pattern for the phase 0 plasmid resulted in expression (**Fig.3**)**.**

Supplementary Figure 3



Supplementary Figure 3. Construction of protein-trap plasmids for three intron phases. (**a**) The multiple cloning sites and surrounding peptide linker coding sequences for constructing protein-trap plasmids in the three intron phases. The following sequence elements are indicated: the “ag” dinucleotide of the 5’ SA site, the sequence encoding the incomplete $(GlyGlySer)_4$ peptide linkers, the *Bam*HI site between the peptide linkers used for sublcloning of protein-trap tags, and the “gt” dinculeotide of the 3’ SD site. (**b**) Each protein-trap tag is amplified as indicated, flanked with a *Bam*H1 site and restriction stuffer at the 5’ end, and a “GGC” trinucleotide followed by a *Bam*HI site and a restriction stuffer at the 3’ end. (**c**) After cloning of the tag into the *Bam*HI site in the three intron phase plasmids, the resulting tag is flanked by flexible $(GlyGlySer)_4$ linkers at either end of the artificial exon.

Supplementary Figure 4



Supplementary Figure 4. Dynamic expression of Wnd during embryogenesis. Expression of *wnd* transcript detected by mRNA *in situ* hybridization (**Left**) and expression of Wnd protein by anti-EGFP staining of a Wnd::EGFP-FlAsH-StrepII-3xFlag protein trap (**Right**) at embryonic stages 8, 11, 12, 14, 16. The mRNA and fusion protein expression patterns are very similar. Bottom right, expression of Wnd as detected with an anti-Wnd antibody at embryonic stages 16 is shown (bottom).

Supplementary Table 1

Plasmid	Description
pBS-SK-attB1-2	General cloning and correction plasmid
pBS-KS-attB1-2-GT-SA	General gene trap plasmid
pBS-KS-attB1-2-GT-SA-mCherry-SV40	mCherry gene trap plasmid
pBS-KS-attB1-2-GT-SA-GAL4-Hsp70pA	GAL4 gene trap plasmid
pBS-KS-attB1-2-GT-SA-Flpo-SV40	Flp gene trap plasmid
pBS-KS-attB1-2-GT-SA-QF-Hsp70	QF gene trap plasmid
pBS-KS-attB1-2-PT-SA-SD-0	General splice phase 0 protein trap plasmid
pBS-KS-attB1-2-PT-SA-SD-0-EGFP-FlAsH-StrepII-TEV-3xFlag	Splice phase 0 EGFP-FlAsH-StrepII-TEV-3xFlag plasmid
pBS-KS-attB1-2-PT-SA-SD-0-mCherry	Splice phase 0 mCherry plasmid
pBS-KS-attB1-2-PT-SA-SD-0-EBFP2-3xMyc	Splice phase 0 EBFP2-3xMyc plasmid
pBS-KS-attB1-2-PT-SA-SD-0-TagRFP-T-3xHA	Splice phase 0 TagRFP-3xHA plasmid
pBS-KS-attB1-2-PT-SA-SD-0-HRP-S	Splice phase 0 HRP-S plasmid
pBS-KS-attB1-2-PT-SA-SD-0-Dendra-V5	Splice phase 0 Dendra-V5 plasmid
pBS-KS-attB1-2-PT-SA-SD-0-KillerRed-V5	Splice phase 0 KillerRed-V5 plasmid
pBS-KS-attB1-2-PT-SA-SD-1	General splice phase 1 protein trap plasmid
pBS-KS-attB1-2-PT-SA-SD-1-EGFP-FlAsH-StrepII-TEV-3xFlag	Splice phase 1 EGFP-FlAsH-StrepII-TEV-3xFlag plasmid
pBS-KS-attB1-2-PT-SA-SD-1-mCherry	Splice phase 1 mCherry plasmid
pBS-KS-attB1-2-PT-SA-SD-1-EBFP2-3xMyc	Splice phase 1 EBFP2-3xMyc plasmid
pBS-KS-attB1-2-PT-SA-SD-1-TagRFP-T-3xHA	Splice phase 1 TagRFP-3xHA plasmid
pBS-KS-attB1-2-PT-SA-SD-1-HRP-S	Splice phase 1 HRP-S plasmid
pBS-KS-attB1-2-PT-SA-SD-1-Dendra-V5	Splice phase 1 Dendra-V5 plasmid
pBS-KS-attB1-2-PT-SA-SD-1-KillerRed-V5	Splice phase 1 KillerRed-V5 plasmid
pBS-KS-attB1-2-PT-SA-SD-2	General splice phase 2 protein trap plasmid
pBS-KS-attB1-2-PT-SA-SD-2-EGFP-FlAsH-StrepII-TEV-3xFlag	Splice phase 2 EGFP-FlAsH-StrepII-TEV-3xFlag plasmid
pBS-KS-attB1-2-PT-SA-SD-2-mCherry	Splice phase 2 mCherry plasmid
pBS-KS-attB1-2-PT-SA-SD-2-EBFP2-3xMyc	Splice phase 2 EBFP2-3xMyc plasmid
pBS-KS-attB1-2-PT-SA-SD-2-TagRFP-T-3xHA	Splice phase 2 TagRFP-3xHA plasmid
pBS-KS-attB1-2-PT-SA-SD-2-HRP-S	Splice phase 2 HRP-S plasmid
pBS-KS-attB1-2-PT-SA-SD-2-Dendra-V5	Splice phase 2 Dendra-V5 plasmid
pBS-KS-attB1-2-PT-SA-SD-2-KillerRed-V5	Splice phase 2 KillerRed-V5 plasmid

Supplementary Table 1. Plasmids for RMCE-based tagging in MiMIC insertion lines. Correction plasmid, gene-trap plasmids, and protein-trap plasmids for intron phases 0, 1 and 2 are indicated. Accession numbers for master plasmids indicated in grey are available from GenBank. Sequence information of all components is available in Supplementary Data. All plasmids are available from the Drosophila Genomics Resource Center (https://dgrc.cgb.indiana.edu/).

Supplementary Table 2

MiMIC	# Lines	% of total	% of subclass
Intergenic	1,340	36.9%	
5' upstream	121	3.3%	9.0%
3' downstream	118	3.2%	8.8%
TE	32	0.9%	2.4%
Other intergenic	1,069	29.4%	79.8%
Intragenic	2,293	63.1%	
5' UTR exon	68	1.9%	3.0%
5' UTR intron	467	12.9%	20.4%
Coding exon	344	9.5%	15.0%
Coding intron	1,190	32.8%	51.9%
3' UTR exon	219	6.0%	9.6%
3' UTR intron	5	0.1%	0.2%
Total	**3,633**		

Supplementary Table 2. Association of MiMIC insertions with gene annotation features. 63% of mapped MiMIC insertions are located within annotated genes (FlyBase r5.32), and the remainder are in intergenic regions. “5’ upstream” and “3’ downstream” are insertions within 500 bp of the 5’ or 3’ end of a gene, respectively. “TE” are insertions that can be mapped to unique sites within transposable elements.

Supplementary Table 3

Rfx	MI00053	Rfx49	Rfx253	7636
MI00053	Lethal			
Rfx49	Escapers			
Rfx253	Escapers	Escapers		
7636	Escapers	Escapers	Escapers	

tutl	MI00290	10979	Tutl4	Tutl23	TutlGAL4	TutlEx383	702
MI00290	Lethal						
10979	NO						
Tutl4	NO	NO					
Tutl23	NO	NO	NO				
TutlGAL4	NO	NO	NO	NO			
TutlEx383	NO	NO	NO	FEW	NO		
702	NO	NO	NO	NO	NO	NO	

comm	MI00380	A490	Δe39	17644	2992	5461
MI00380	Lethal					
A490	NO					
Δe39	NO	NO				
17644	NO	FEW	NO			
2992	NO	NO	NO	NO		
5461	NO	NO	NO	NO	NO	

wnd	MI00494	Wnd1	Wnd2	Wnd3	5584	7942
MI00494	Lethal					
Wnd1	YES					
Wnd2	YES	YES				
Wnd3	YES	YES	YES			
5584	YES	YES	YES	YES		
7942	YES	YES	YES	YES	YES	

Supplementary Table 3. Complementation analysis of lethal MiMIC insertion lines. Results of complementation analysis are indicated for *Mi{MIC}Rfx*^{MI00053} and previously described *Rfx* mutant alleles (see Online Methods), *Mi{MIC}tutl*^{MI00290} and other *tutl* alleles (see Online Methods), *Mi{MIC}comm*^{MI00380} and other *comm* alleles (see Online Methods), and *Mi{MIC}wnd*^{MI00494} and other *wnd* alleles (see Online Methods). Failure to complement (NO); escapers illustrate a phenotype previously described for Rfx mutations (Escapers), and a few escapers without an obvious phenotype (FEW).

Supplementary Table 4

Gene	MiMIC	Gene trap	Lethality	RMCE			
				Casette	Total	Expression	%
gogo	**MI00065**	NO	V	**GAL4-Hsp70**	4	2	50%
Tl	**MI00181**	NO	V	**GAL4-Hsp70**	4	1	25%
				Flp-SV40	6	2	33%
				QF-Hsp70	3	0	0%
caps	**MI00249**	NO	L	**GAL4-Hsp70**	6	1	17%
				Flp-SV40	5	2	40%
				QF-Hsp70	3	0	0%
MYPT-75D	**MI00314**	NO	V	**GAL4-Hsp70**	6	1	17%
				Flp-SV40	6	1	17%
				QF-Hsp70	5	2	40%
BM-40-SPARC	**MI00329**	NO	L	**GAL4-Hsp70**	3	2	67%
				Flp-SV40	6	1	17%
				QF-Hsp70	2	0	0%
Total					59	15	25%

Supplementary Table 4. Summary of gene-trapping experiments. MiMIC insertions in five genes were tagged with different gene-trap cassettes: gene name, MiMIC line identifier, gene-trap status, and associated phenotype, viable (V) or lethal (L), are indicated. Results for three gene-trap cassettes for each gene: total lines, number of expressing lines, percentage of expressing lines. The total numbers of lines and expressing lines, and the average percentage of expressing lines, are indicated.

Supplementary Table 5

Primer	Sequence
pMiLR-Correction-TOP	AGCTACCTTAATCTCAAGAAGAGCAAAACAAAAGCAACTAATGTAACGGAAGCTTGA
pMiLR-Correction-	TAATTTCCCCCGCGGGAATTCAAGCTTCCGTTACATTAGTTGCTTTTGTTTTGCTCTT
attP1-pMiLR-F	CCGAAGCTTCCCAGGTCAGAAGCGGTTTTCGGGAG
attP1-pMiLR-R	CCGCTCGAGGCGGCCGCTCTAGACCCGGGGGATCCAACCCCTTGTGTCATGTCGG
attP2-pMiLR-F	CCGCTCGAGACTAGTCTGCAGGTCGACGAATTCAACCCCTTGTGTCATGTCGGCGA
attP2-pMiLR-R	CCGCCGCGGCCCAGGTCAGAAGCGGTTTTCGGGAG
MHC-SA-XmaI-F	TCCCCCCGGGAGTCGATCCAACATGGCGACT
MHC-SA-EGFP-R	CTCCTCGCCCTTGCTCACCATTTTGTTAGTTAGTTAGACCTGCGGAAGAGAGATAAA
MHC-SA-EGFP-F	GATTTATCTCTCTTCCGCAGGTCTAACTAACTAACAAAATGGTGAGCAAGGGCGAG
EGFP-SpeI-R	GGACTAGTTGATCATAATCAGCCATACCAC
attB1-pBS-F	CCGGAGCTCGCTAGCGATGTAGGTCACGGTCTCGAAG
attB1-pBS-R	CCGGAATTCCTGCAGACTAGTTCTAGAGTCGACCATCATGATGGACCAGATGGGTG
attB2-pBS-F	CCGGAATTCCTCGAGGGATCCAAGCTTGTCGACCATCATGATGGACCAGATGGGTG
attB2-pBS-R	CCGGGTACCATGCATGATGTAGGTCACGGTCTCGAAG
SA-XbaI-F	GCTCTAGAAGTCGATCCAACATGGCGAC
SA-PstI-R	AACTGCAGGACCTGCGGAAGAGAGATAAATC
GAL4-Hsp70-EcoRI-F	GGAATTCTAACTAACTAACAAAATGAAGCTACTGTCTTCTATCGAAC
GAL4-Hsp70-BamHI-R	CGCGGATCCGGCCGCTCTAGAACTAGTGGATC
QF-SV40-EcoRI-F	GGAATTCTAACTAACTAACAAAATGCCGCCTAAACGCAAGACACTC
QF-SV40-BamHI-R	CGCGGATCCGATCCAGACATGATAAGATACATTG
Flpo-SV40-EcoRI-F	GGAATTCTAACTAACTAACAAAATGAGCCAGTTCGACATCCTG
Flpo-SV40-BamHI-R	CGCGGATCCGATCCAGACATGATAAGATACATTG
SA-SD-Phase-0-R	CGCGGATCCGCCGCTACCTCCGCTTCCACCGGAACCTCCCACCTGCGGAAGAGAG
SA-SD-Phase-0-F	CGCGGATCCGGAGGTAGCGGTGGAAGCGGAGGTTCCGGCGAGGTAAGTTATTGAA
SD-HindIII-R	CCCAAGCTTAGAAGTTCAAATGGGCTTTC
SA-SD-Phase-1-R	CGCGGATCCGCCGCTACCTCCGCTTCCACCGGAACCTCCGCCACCTGCGGAAGAG
SA-SD-Phase-1-F	CGCGGATCCGGAGGTAGCGGTGGAAGCGGAGGTTCCGGAGGTAAGTTATTGAACA

SA-SD-Phase-2-R	CGCGGATCCGCCGCTACCTCCGCTTCCACCGGAACCTCCCGACCTGCGGAAGAGA
SA-SD-Phase-2-F	CGCGGATCCGGAGGTAGCGGTGGAAGCGGAGGTTCCGGCAGGTAAGTTATTGAAC
EGFPmultiFINAL-F	CGCGGATCCGTGTCCAAGGGCGAGGAGCTG
EGFPmultiFINAL-R	CGCGGATCCGCCCTTGTCGTCATCGTCCTTGTAATC
Cherry-F	CGCGGATCCGTGAGCAAGGGCGAGGAGGATAAC
Cherry-R	CGCGGATCCGCCCTTGTACAGCTCGTCCATG
EBFP2-Myc-F	CGCGGATCCGTGTCCAAGGGCGAGGAGCTGTTC
EBFP2-Myc-R	CGCGGATCCGCCGAGATCCTCCTCCGAGATCAG
TagRFP-HA-F	CGCGGATCCGTGTCCAAGGGCGAGGAGCTGATC
TagRFP-HA-R	CGCGGATCCGCCAGCGTAGTCTGGCACGTCGTAG
HRP-S-F	CGCGGATCCCAGCTGACCCCCACCTTCTACGATAAC
HRP-S-R1	TCGAACTTAGCGGCTGCGGTCTCCTTGCCGCTTCCGCTGTTGCTGTTCACCACGCG
HRP-S-R2	CGCGGATCCGCCGCTATCCATGTGCTGGCGCTCGAACTTAGCGGCTGCGGTC
Dendra-V5-F	CGCGGATCCAACACCCCCGGCATCAACCTGATC
Dendra-V5-R1	AGCAGTGGATTCGGGATCGGCTTGCCGCCGCTTCCCCACACCTGGCTGGGCAGGG
Dendra-V5-R2	CGCGGATCCGCCGGTGCTATCCAGTCCGAGCAGTGGATTCGGGATCGGCTTG
KillerRed-V5-F	CGCGGATCCGGCAGCGAGGGCGGACCAGCCCTG
KillerRed-V5-R1	AGTCCGAGCAGTGGATTCGGGATCGGCTTGCCGCCGCTTCCATCCTCATCGGAGC
KillerRed-V5-R2	CGCGGATCCGCCGGTGCTATCCAGTCCGAGCAGTGGATTCGGGATC
mCherry-Seq-F	ACGGCGAGTTCATCTACAAG
mCherry-Seq-R	TTCAGCCTCTGCTTGATCTC
EGFPdo-Seq-F	GGATGACGGCACCTACAAGAC
EGFPdo-Seq-R	GTGGCTGTTGAAGTTGTACTC
EBFP2do-Seq-F	GGACGACGGCACCTACAAGAC
EBFP2do-Seq-R	GAAGTTGTACTCCAGCTTGTG
TagRFPdo-Seq-F	GGCTGCCTGATCTACAACGTG
TagRFPdo-Seq-R	GGGTACAGCATCTCGGTGTTG
Hrpdo-Seq-F	AAGGATAGCTTCCGCAACGTG
Hrpdo-Seq-R	GCTGAAGTTGTACAGGCGATC

Dendrado-Seq-F	GATAAGGGCATCTGCACCATC
Dendrado-Seq-R	ACGTGCAGCTTCTCGGTGCTG
Killerreddo-Seq-F	CTACGAGCTGGATGATACCTG
Killerreddo-Seq-R	CAGGATATCGACCAGCTGATC
GAL4-1R	TGATGAGCTGCCGAGTCAATC
GAL4-5F	AACTGTGCATCGTGCACCATC
FLP0-Seq-R	AGTAGATGTGCCTGGACACGCTTG
SV40pA-Long-F	TCCAACCTATGGAACTGATGAATG
QF-Seq-R1	TTGGCCGTCTTCAGATGGATCTTG
Hsp70-pA-Alt-F	GTCTATCGATACCGTCGACTAAAG
Orientation-MiL-F	GCGTAAGCTACCTTAATCTCAAGAAGAG
Orientation-MiL-R	CGCGGCGTAATGTGATTTACTATCATAC
Wnd-F	CGGTGTGGTGCTATGGGAAAT
Wnd-R	GGCACGTCGATGGGCTGAGAT

Supplementary Table 5. Primers. Primers and sequences used in this study.

Supplementary Table 6

phiC31 MIMIC injections: OVERVIEW Phase 0 Swapping						
Inj	MI00296/Rhea (3)			MI00393/CadN (2)		
	Fertility	Trans	%	Fertility	Trans	%
COR-SK	61	28	45.9%	22	8	36.4%
A-0	50	34	68.0%	16	14	87.5%
A-1	39	25	64.1%	16	10	62.5%
A-2	40	14	35.0%	11	3	27.3%
C-0	53	21	39.6%	8	2	25.0%
D-0	55	28	50.9%	13	6	46.2%
E-0	49	28	57.1%	13	6	46.2%
F-0	18	9	50.0%	15	9	60.0%
G-0	100	39	39.0%	15	8	53.3%
H-0	43	22	51.2%	21	11	52.4%

phiC31 MIMIC injections: OVERVIEW Phase 1 Swapping									
Inj	MI00053/Rfx (3)			MI00290/tutl (2)			MI00380/Comm (3)		
	Fertility	Trans	%	Fertility	Trans	%	Fertility	Trans	%
COR-SK	3	2	66.7%	9	4	44.4%	33	17	51.5%
A-0	30	17	56.7%	20	12	60.0%	13	9	69.2%
A-1	5	2	40.0%	11	5	45.5%	18	12	66.7%
A-2	5	2	40.0%	20	13	65.0%	32	13	40.6%
C-1	14	2	14.3%	17	6	35.3%	18	7	38.9%
D-1	11	5	45.5%	9	5	55.6%	14	7	50.0%
E-1	18	6	33.3%	4	2	50.0%	24	13	54.2%
F-1	16	8	50.0%	18	9	50.0%	21	15	71.4%
G-1	41	17	41.5%	8	6	75.0%	25	10	40.0%
H-1	8	4	50.0%	10	5	50.0%	12	6	50.0%

phiC31 MIMIC injections: OVERVIEW Phase 2 Swapping			
Inj	MI00494/wnd (3)		
	Fertility	Trans	%
COR-SK	3	0	0.0%
A-0	18	7	38.9%
A-1	12	1	8.3%
A-2	14	5	35.7%
C-2	10	4	40.0%
D-2	10	6	60.0%
E-2	8	5	62.5%
F-2	9	6	66.7%
G-2	7	3	42.9%
H-2	3	1	33.3%

phiC31 MIMIC injections: OVERVIEW 5'UTR Swapping															
Inj	MI00065/gogo (3)			MI00181/Tl (3)			MI00249/CAPS (3)			MI00314/MYPT-75D (3)			MI00329/BM-40-SPARC (3)		
	Fertility	Trans	%	Fertility	Trans	%	Fertility	Trans	%	Fertility	Trans	%	Fertility	Trans	%
2	12	6	50.0%	37	4	10.8%	14	9	64.3%	62	22	35.5%	15	3	20.0%
3	6	0	0.0%	29	9	31.0%	14	9	64.3%	30	8	26.7%	12	6	50.0%
4	5	0	0.0%	26	3	11.5%	10	3	30.0%	38	5	13.2%	15	2	13.3%

Supplementary Table 6. Efficiency of RMCE conversion. Fertile vials (Fertility), vials with transgenic progeny scored by loss of the *yellow*$^+$ marker of MiMIC (Trans) and RMCE efficiencies are indicated. RMCE efficiencies for protein-trapping experiments in intron phases 0, 1 and 2 for MiMIC insertions in coding introns, and for enhancertrapping experiments using MiMIC insertions in 5’ UTR introns, are indicated. Plasmids used are the correction plasmid (COR-SK); the GAL4 (2), Flp (3), and QF (4) gene trap plasmids; and the EGFP-FlAsH-StrepII-3xFlag (A), mCherry (C), EBFP2-3xMyc (D), TagRFP-3xHA (E), HRP-S (F), Dendra-V5 (G) and Killer Red-V5 (H) protein-trap plasmids. Intron phases of the protein trap plasmids are indicated (0, 1 or 2).

Supplementary Data. Plasmid inserts. Sequences of gene trap inserts and protein trap inserts.

GENE TRAP INSERTS

pBS-KS-attB1-2-GT-SA-GAL4-Hsp70pA *Eco*RI/*Bam*HI insert

GAL4 ORF

```
gaattcTAACTAACTAACAAAATGAAGCTACTGTCTTCTATCGAACAAGCATGCGATATTTGCC
GACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCGCCAAGTGTCTGAAGAACAACTG
GGAGTGTCGCTACTCTCCCAAAACCAAAAGGTCTCCGCTGACTAGGGCACATCTGACAGAAGTG
GAATCAAGGCTAGAAAGACTGGAACAGCTATTTCTACTGATTTTTCCTCGAGAAGACCTTGACA
TGATTTTGAAAATGGATTCTTTACAGGATATAAAAGCATTGTTAACAGGATTATTTGTACAAGA
TAATGTGAATAAAGATGCCGTCACAGATAGATTGGCTTCAGTGGAGACTGATATGCCTCTAACA
TTGAGACAGCATAGAATAAGTGCGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGAC
AGTTGACTGTATCGATTGACTCGGCAGCTCATCATGATAACTCCACAATTCCGTTGGATTTTAT
GCCCAGGGATGCTCTTCATGGATTTGATTGGTCTGAAGAGGATGACATGTCGGATGGCTTGCCC
TTCCTGAAAACGGACCCCAACAATAATGGGTTCTTTGGCGACGGTTCTCTCTTATGTATTCTTC
GATCTATTGGCTTTAAACCGGAAAATTACACGAACTCTAACGTTAACAGGCTCCCGACCATGAT
TACGGATAGATACACGTTGGCTTCTAGATCCACAACATCCCGTTTACTTCAAAGTTATCTCAAT
AATTTTCACCCCTACTGCCCTATCGTGCACTCACCGACGCTAATGATGTTGTATAATAACCAGA
TTGAAATCGCGTCGAAGGATCAATGGCAAATCCTTTTTAACTGCATATTAGCCATTGGAGCCTG
GTGTATAGAGGGGGAATCTACTGATATAGATGTTTTTTACTATCAAAATGCTAAATCTCATTTG
ACGAGCAAGGTCTTCGAGTCAGGTTCCATAATTTTGGTGACAGCCCTACATCTTCTGTCGCGAT
ATACACAGTGGAGGCAGAAAACAAATACTAGCTATAATTTTCACAGCTTTTCCATAAGAATGGC
CATATCATTGGGCTTGAATAGGGACCTCCCCTCGTCCTTCAGTGATAGCAGCATTCTGGAACAA
AGACGCCGAATTTGGTGGTCTGTCTACTCTTGGGAGATCCAATTGTCCCTGCTTTATGGTCGAT
CCATCCAGCTTTCTCAGAATACAATCTCCTTCCCTTCTTCTGTCGACGATGTGCAGCGTACCAC
AACAGGTCCCACCATATATCATGGCATCATTGAAACAGCAAGGCTCTTACAAGTTTTCACAAAA
ATCTATGAACTAGACAAAACAGTAACTGCAGAAAAAAGTCCTATATGTGCAAAAAAATGCTTGA
TGATTTGTAATGAGATTGAGGAGGTTTCGAGACAGGCACCAAAGTTTTTACAAATGGATATTTC
CACCACCGCTCTAACCAATTTGTTGAAGGAACACCCTTGGCTATCCTTTACAAGATTCGAACTG
AAGTGGAAACAGTTGTCTCTTATCATTTATGTATTAAGAGATTTTTTCACTAATTTTACCCAGA
AAAAGTCACAACTAGAACAGGATCAAAATGATCATCAAAGTTATGAAGTTAAACGATGCTCCAT
CATGTTAAGCGATGCAGCACAAAGAACTGTTATGTCTGTAAGTAGCTATATGGACAATCATAAT
GTCACCCCATATTTTGCCTGGAATTGTTCTTATTACTTGTTCAATGCAGTCCTAGTACCCATAA
AGACTCTACTCTCAAACTCAAAATCGAATGCTGAGAATAACGAGACCGCACAATTATTACAACA
AATTAACACTGTTCTGATGCTATTAAAAAAACTGGCCACTTTTAAAATCCAGACTTGTGAAAAA
TACATTCAAGTACTGGAAGAGGTATGTGCGCCGTTTCTGTTATCACAGTGTGCAATCCCATTAC
CGCATATCAGTTATAACAATAGTAATGGTAGCGCCATTAAAAATATTGTCGGTTCTGCAACTAT
CGCCCAATACCCTACTCTTCCGGAGGAAAATGTCAACAATATCAGTGTTAAATATGTTTCTCCT
GGCTCAGTAGGGCCTTCACCTGTGCCATTGAAATCAGGAGCAAGTTTCAGTGATCTAGTCAAGC
TGTTATCTAACCGTCCACCCTCTCGTAACTCTCCAGTGACAATACCAAGAAGCACACCTTCGCA
TCGCTCAGTCACGCCTTTTCTAGGGCAACAGCAACAGCTGCAATCATTAGTGCCACTGACCCCG
TCTGCTTTGTTTGGTGGCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCT
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TCACTTTCACTAACAGTAGCAACGGTCCGAACCTCATAACAACTCAAACAAATTCTCAAGCGCT
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AACCCCGCAAGTTCACTTCAACTGTGCATCGTGCACCATCTCAATTTCTTTCATTTATACATCG
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AAATATATTACGAGGGCTTATTCAGAAGCTTATCGATACCGTCGACTAAAGCCAAATAGAAATT
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TGTAAATAACTAATACATAATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATT
AGCTACTTGGTTAATAAATAGAATATATTTATTTAAAGATAATTCGTTTTTATTGTCAGGGAGT
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pBS-KS-attB1-2-GT-SA-Flpo-SV40 *Eco*RI/*Bam*HI insert

Flpo ORF

```
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TCAAGTACAAGACCCAGAAGGCCACCATCCTGGAGGCCAGCCTGAAGAAGCTGATCCCCGCCTG
GGAGTTCACCATCATCCCTTACAACGGCCAGAAGCACCAGAGCGACATCACCGACATCGTGTCC
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CCTGGTGTCCAGGTACTACGCCTACGACCCCATCAGCAAGGAGATGATCGCCCTGAAGGACGAG
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CCCCAAGGACTTTCCTTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACT
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CTTATCATGTCTGGATCggatcc
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pBS-KS-attB1-2-GT-SA-QF-Hsp70 *Eco*RI/*Bam*HI insert

QF ORF

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GTCCCCATGGCCAATTTCGGATATTCGACTGTAGACTATGATGCGATGGTGGATGATCTGGCAT
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PROTEIN TRAP INSERTS

pBS-KS-attB1-2-PT-SA-SD-0/1/2-EGFP-FlAsH-StrepII-TEV-3xFlag *Bam*HI insert

GFP
FlAsH
StrepII
TEV
3xFlag

```
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ACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGATCACATGAAGCAGCACGATTTCT
TCAAGAGCGCCATGCCCGAGGGCTACGTGCAGGAGCGCACCATCAGCTTCAAGGATGACGGCAC
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CCGGGAAGCGGCAACTGGTCCCACCCCCAGTTCGAGAAGGAAAACCTGTACTTCCAGAGCGATT
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Cggatcc
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pBS-KS-attB1-2-PT-SA-SD-0/1/2-mCherry *Bam*HI insert

mCherry

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ggatccGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
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GTACAAGGGCggatcc
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pBS-KS-attB1-2-PT-SA-SD-0/1/2-EBFP2-3xMyc *Bam*HI insert

EBFP2
3xMyc

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ACCCTGAGCCACGGCGTGCAGTGCTTCGCCCGCTACCCCGATCACATGAAGCAGCACGATTTCT
TCAAGAGCGCCATGCCCGAGGGCTACGTGCAGGAGCGCACCATTTTCTTCAAGGACGACGGCAC
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ACAACATCTACATCATGGCCGTGAAGCAGAAGAACGGCATCAAGGTCAACTTCAAGATCCGCCA
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ACGAGAAGCGCGATCACATGGTGCTGCTGGAGTTCCGCACCGCCGCCGGCATCACCCTGGGCAT
GGATGAGCTGTACAAGGGAAGCGGCGAGCAAAAGCTCATCAGCGAGGAAGATCTGGAACAGAAG
TTGATTTCCGAAGAGGACTTGGAGCAGAAACTGATCTCGGAGGAGGATCTCGGCggatcc

pBS-KS-attB1-2-PT-SA-SD-0/1/2-TagRFP-T-3xHA *Bam*HI insert

TagRFP
3xHA

```
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ACCTCCTTCATGTACGGCAGCCGCACCTTCATCAACCACACCCAGGGCATCCCCGATTTCTTCA
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CAAGCTGAACGGCATGGATGAGCTGTACAAGGGAAGCGGCTACCCATACGATGTGCCCGATTAC
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cc
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pBS-KS-attB1-2-PT-SA-SD-0/1/2-HRP-S *Bam*HI insert

HRP
S

ggatccCAGCTGACCCCCACCTTCTACGATAACAGCTGCCCCAACGTGTCCAACATCGTGCGCG
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pBS-KS-attB1-2-PT-SA-SD-0/1/2-Dendra-V5 *Bam*HI insert

Dendra
V5

```
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<u>pBS-KS-attB1-2-PT-SA-SD-0/1/2-KillerRed-V5 *Bam*HI insert</u>

Killerred
V5

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