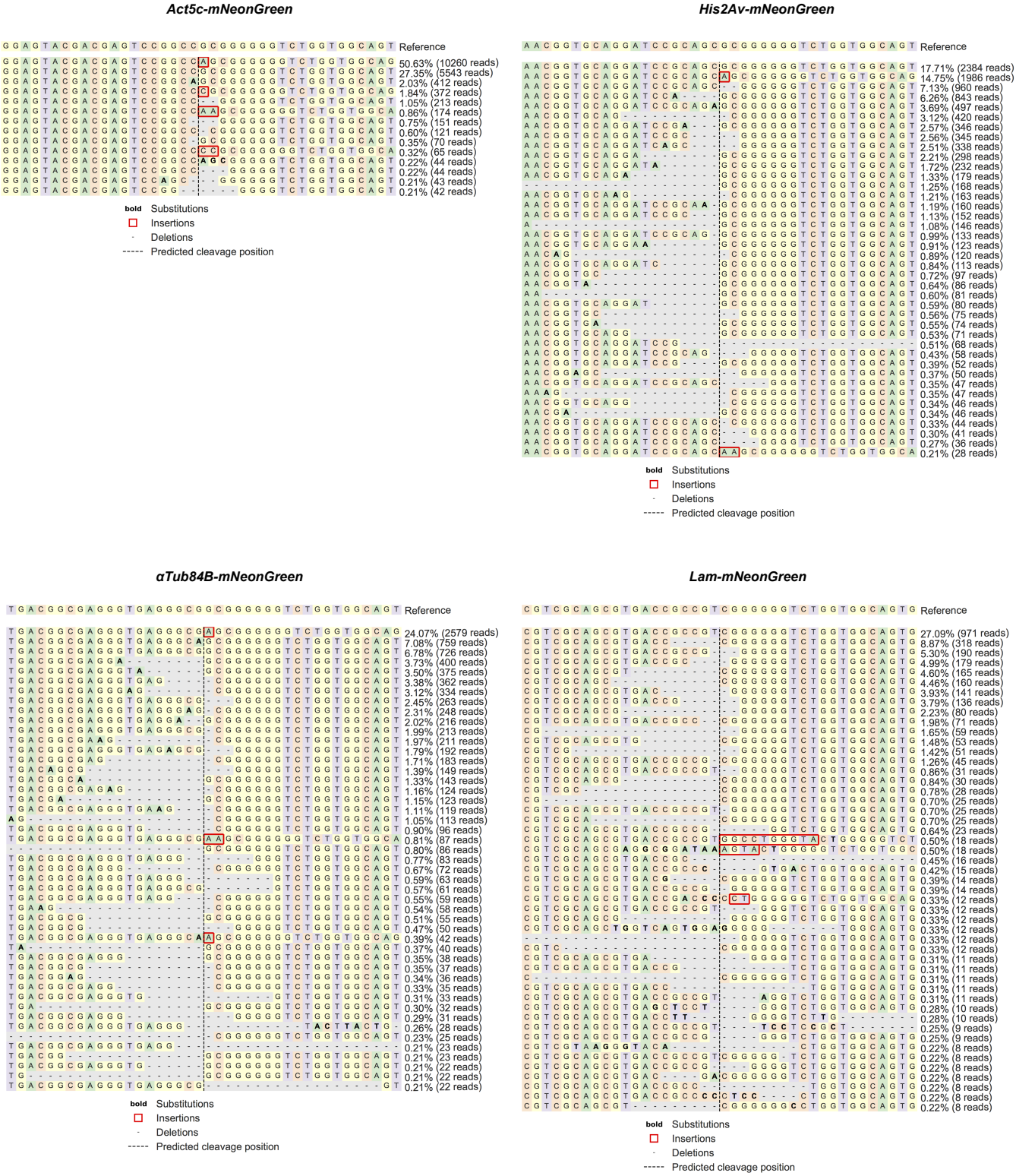
Supplemental Figure 1

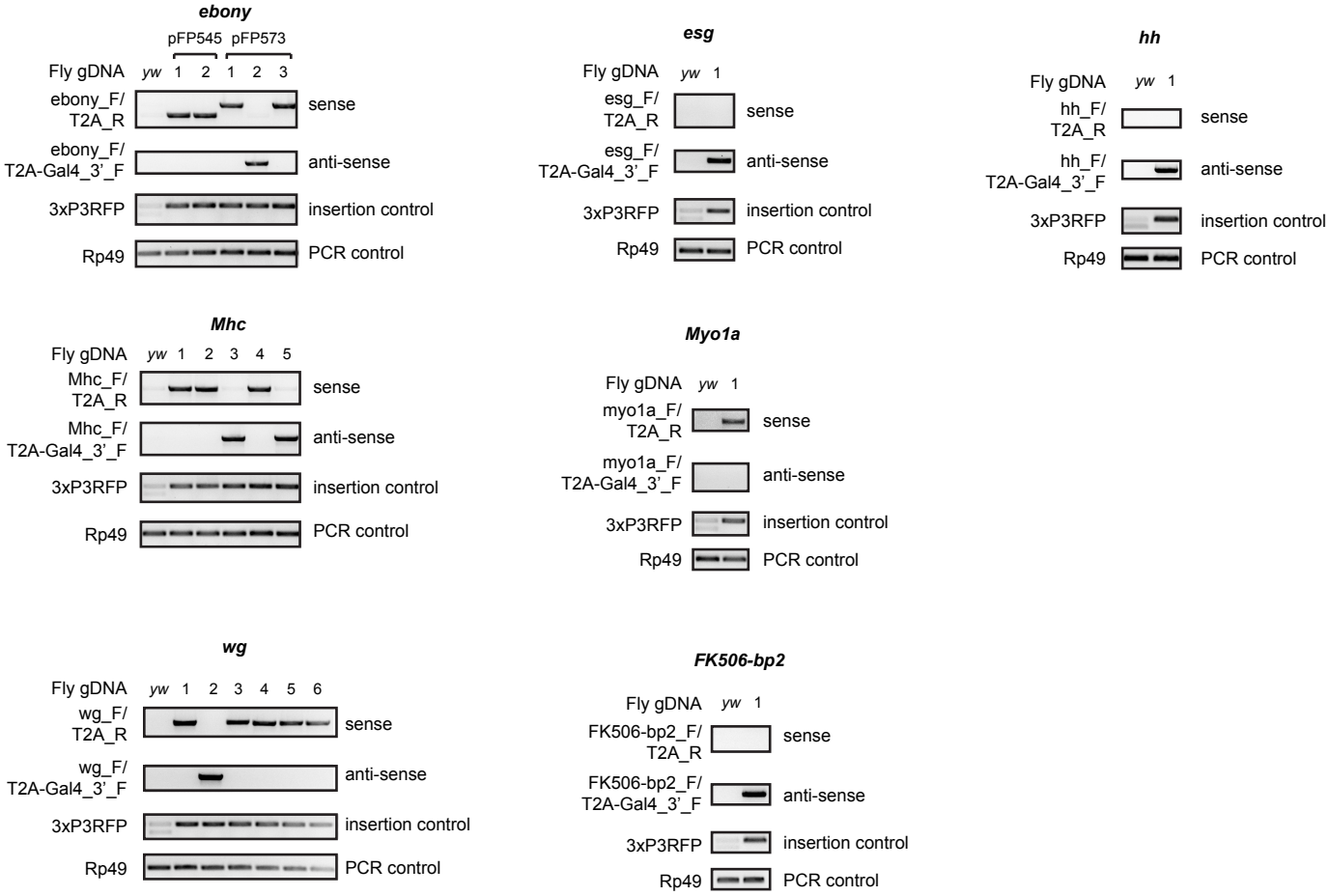


Supplemental Figure 1 – Next-generation sequencing analysis of mNeonGreen insertion sites in transfected S2R+ cells using CRISPresso2

Supplemental Figure 2

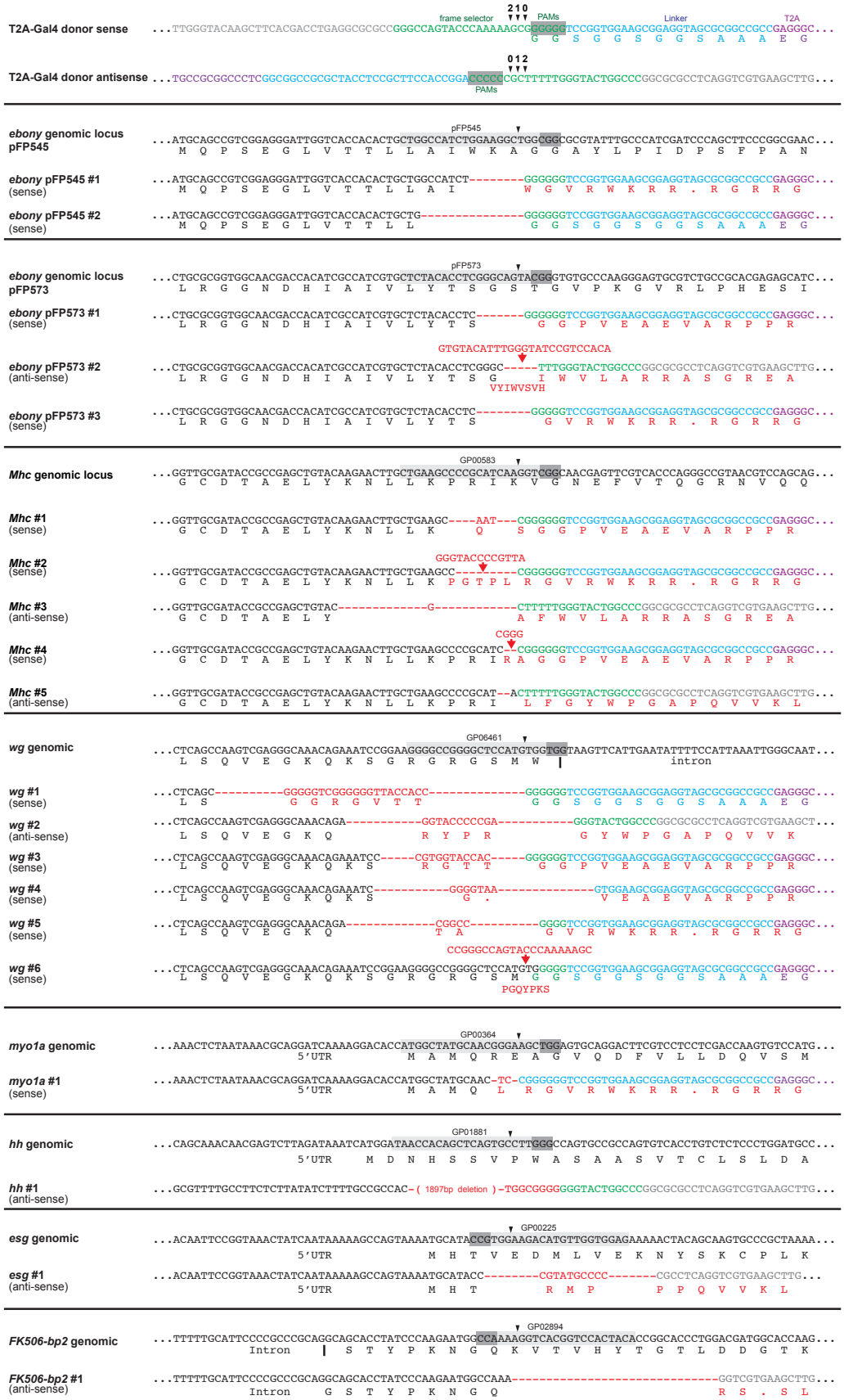
Sense insertion

Antisense insertion



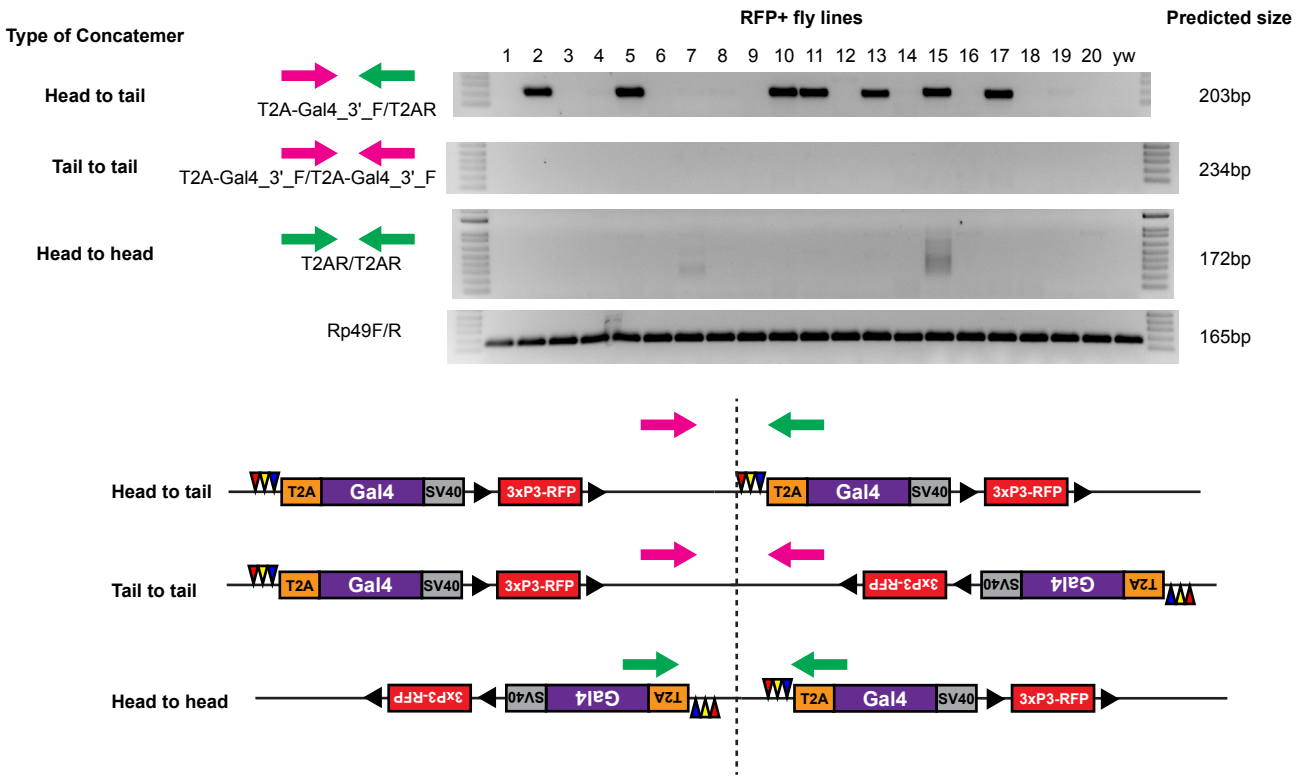
Supplemental Figure 2 – Diagnostic PCR of *CRISPaint-T2A-Gal4* insertions in RFP+ fly lines to confirm their insertion site and orientation.

Supplemental Figure 3



Supplemental Figure 3 – Sequence structure of *CRISPaint-T2A-Gal4* insertions in RFP+ fly lines

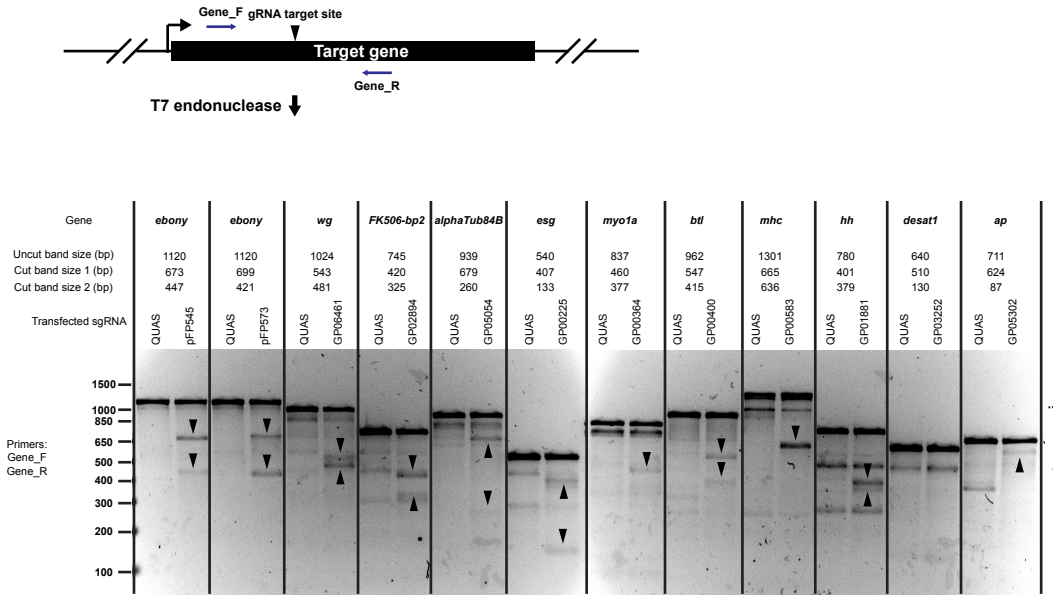
Supplemental Figure 4



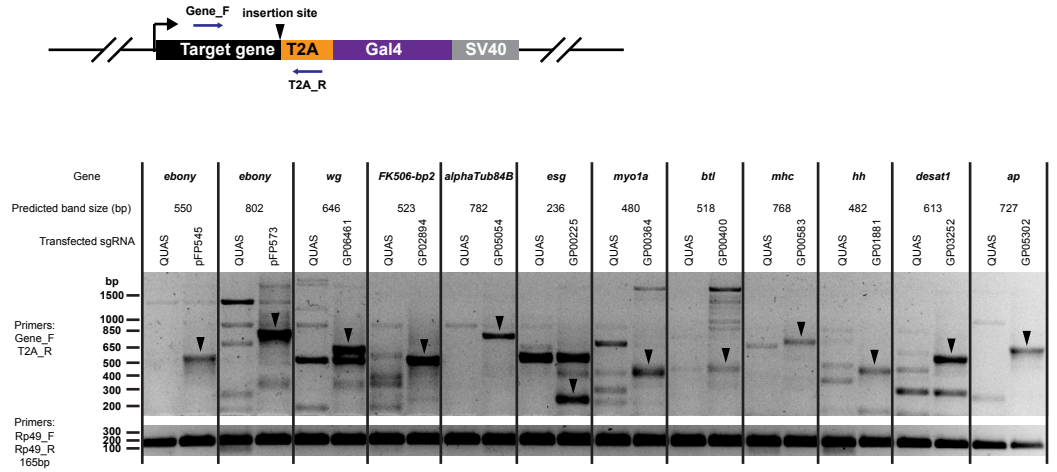
Supplemental Figure 4 – Diagnostic PCR of *CRISPaint-T2A-Gal4* insertions in RFP+ fly lines to detect concatemers

Supplemental Figure 5

A

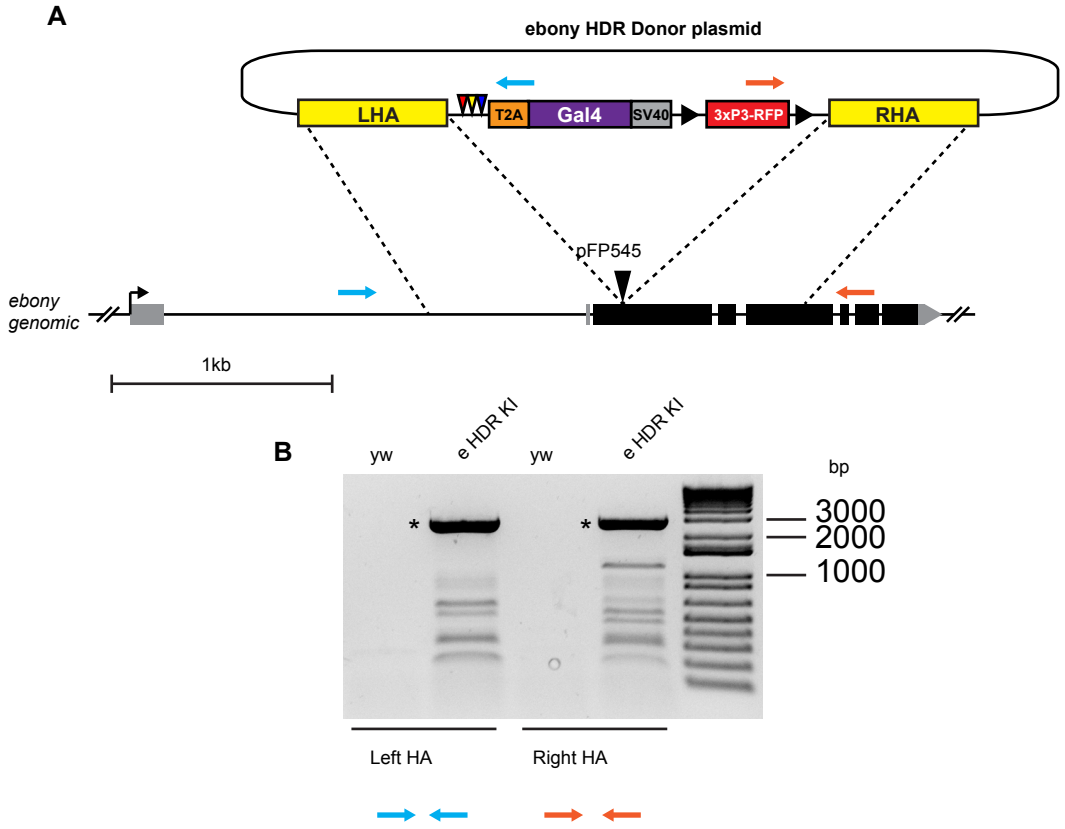


B



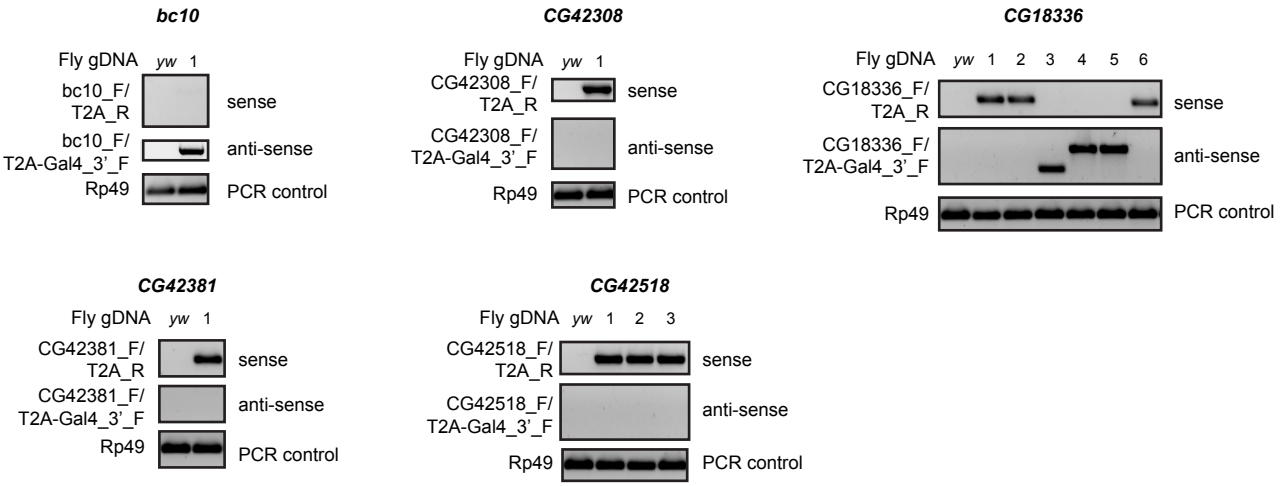
Supplemental Figure 5 – Cutting efficiency of 12 sgRNAs in transfected S2R+ cells. (**A**) T7 endonuclease assay. (**B**) Diagnostic PCR to detect for presence of sense orientation *CRISPaint-T2A-Gal4* insertion events.

Supplemental Figure 6



Supplemental Figure 6 – Construction of *ebony-Gal4* HDR insertion. (**A**) Targeting strategy of *ebony* HDR donor plasmid and *ebony* genomic locus. (**B**) Diagnostic PCR of *ebony-Gal4* HDR insertion. Asterisks indicate correct size band.

Supplemental Figure 7



Supplemental Figure 7 – Diagnostic PCR of CRISPaint-T2A-Gal4 insertions in smORF genes

	Gene and sgRNA information			
Gene Name	**Protein localization**	**sgRNA plasmid (TRiP)**	**sgRNA target site with PAM**	**frame selector sgRNA**
Act5c	actin	GP07595	GCGGTGCACAATGGAGGGGCCGG	2
His2Av	chromatin	GP07596	GGTGCAGGATCCGCAGCGGAAGG	2
αTub84B	microtubules	GP07609	CGGCGAGGGTGAGGGCGCTGAGG	2
Lamin	nuclear envelope	GP07612	CGCAGCGTGACCGCCGTGGACGG	1
Untransfected				

	CRISPresso2 output			
Gene Name	**seamless**	**in-frame in/del**	**frameshift in/del**	**% in-frame**
Act5c	5543	1343	13068	34.50937155
His2Av	2384	2620	8279	37.6722126
αTub84B	726	3034	6901	35.26873652
Lamin	971	801	1757	50.21252479
Untransfected				

	FACs Transfected			FACs Puro-selected		
Gene Name	**mNeonGreen+**	**Single cells total**	**% mNeonGreen+**	**mNeonGreen+**	**Single cells total**	**% mNeonGreen**
Act5c	1963	80589	2.435816302	45355	50883	89.1358607
His2Av	1554	80291	1.935459765	32780	46298	70.80219448
αTub84B	150	78752	0.190471353	16682	52643	31.6889235
Lamin	385	81688	0.471305455	34826	49234	70.73567047
Untransfected	2	83869	0.002384671	10	55656	0.017967515

	Cell counting transfected (n=6)			Cell counting Puro-selected (n=3)		
Gene Name	**mNeonGreen+**	**Total cells**	**Average % mNeonGreen+**	**mNeonGreen+**	**Total cells**	**Average % mNeonGreen+**
Act5c	17/17/26/18/22/25	571/679/577/599/574/613	3.5	342/280/318	397/398/377	80.3
His2Av	26/21/12/19/13/19	634/634/568/525/669/612	3	314/327/315	397/402/400	79.7
αTub84B				412/400/403	412/400/403	100
Lamin				270/282/259	382/388/374	70.9
Untransfected						

Supplemental Table 1 – Quantification of CRISPaint-mNeonGreen insertion events in transfected and puro-selected S2R+ cells by CRISPresso2, FACs, and cell counting of confocal images.

					endogenous gene			
Clone name	**Gene-mNeonGreen**	**predicted mNeonGreen fusion (kDa)**	**Observed Localization**	**mNeon insertion site**	**allele 1**	**allele 2**	**allele 3**	**allele 4**
A3	Act5c	71.4	very infrequent small rods	seamless	1bp deletion	1bp insertion		
A5	Act5c	71.4	very frequent long rods	seamless	numerous in/dels			
A19	Act5c	71.4	no rods, cyto and nuclear	N/A	numerous in/dels			
B1	His2Av	44.5	cyto and nuclear	N/A	4bp deletion	19bp deletion		
B11	His2Av	44.5	nuclear	seamless	3bp deletion	2bp deletion	1bp deletion	27bp deletion
B14	His2Av	44.5	nuclear	3 bp deletion, 1bp change	19bp deletion	3bp deletion	1bp deletion	
C2	alphatub84B	79.5	mitotic spindles	seamless	wt	6bp deletion	1bp deletion	
C6	alphatub84B	79.5	mitotic spindles	seamless	insertion of 1482bp from alphatub84D			
C13	alphatub84B	79.5	mitotic spindles	3bp deletion	wt	6bp insertion		
D1	Lam	100.9	cytoplasmic, nuclear	N/A	8bp deletion	4bp deletion	3bp insertion	
D6	Lam	100.9	nuclear lamina	seamless	3bp deletion			
D9	Lam	100.9	nuclear lamina, polarized	seamless	N/A			

Supplemental Table 2 – Molecular characterization of single cell cloned mNeonGreen-expressing S2R+ lines.

Gene	Chr.	target gene sgRNA	target site (no PAM)	Frame selector sgRNA	# G0 crosses	# G0 founders	% founders/total	# G1 progeny screened	# G1 RFP+ progeny	% RFP G1/total G1
Mhc	2	GP00583	CTGAAGCCCCGCATCAAGGT	1	29	6	21	2086	21	1.01
ebony	3	pFP545	TGGCCATCTGGAAGGCTGG	1	16	3	19	1298	11	0.85
FK506-bp2	2	GP02894	TGTAGTGGACCGTGACCTTT	1	13	2	15	901	2	0.22
wg	2	GP06461	GGGGCCGGGGCTCCATGTGG	0	40	6	15	4423	26	0.59
ebony	3	pFP573	TCTACACCTCGGGCAGTAC	1	23	3	13	2121	27	1.27
esg	2	GP00225	CTCCACCAACATGTCTTCCA	2	15	1	7	772	1	0.13
Myo1a	2	GP00364	ATGGCTATGCAACGGGAAGC	1	17	1	6	860	1	0.12
hh	3	GP01881	TAACCACAGCTCAGTGCCTT	2	39	2	5	1975	3	0.15
alphaTub84B	3	GP05054	GCTGACGGTAGGTTCCGGTA	1	6	0	0	506	0	0.00
btl	2	GP00400	GGCAAAAGTGCCGATCACGC	2	26	0	0	2085	0	0.00
Desat1	3	GP03252	AAGCTGCAGGAGGACTCCAC	1	7	0	0	386	0	0.00
ap	2	GP05302	CACCACCTGTAGCACATCAA	0	29	0	0	2928	0	0.00

Supplemental Table 3 – Germ line knock-in efficiency of *CRISPaint-T2A-Gal4*

#	Gene targeted	sgRNA	G0 cross #	Head-tail insertion by PCR?	Splinkerette 5' (PCR Purification)	Splinkerette 5' (Gel Purification)	Splinkerette 3' (PCR Purification)	Splinkerette 3' (Gel Purification)	Insert orientation	Insert frame	Insertion site sequence	Gal4 expression	Homozygous phenotype	Complementation test
1	ebony	pFP545	1	no	ebony 5' insertion site	no bands	ebony 3' insertion site	ebony 3' insertion site	Sense	Out	8bp del	none	dark cuticle pigment	dark cuticle pigment over e1 on TM3
2	ebony	pFP545	2	yes	ebony 3' insertion site	Band 1: ebony 3' insertion site Band 2: 3' end of donor plasmid	5' end of donor plasmid	5' end of donor plasmid	Sense	In	15bp del	anterior and posterior trachae in larvae, whole body cuticle in pupae and adult	lethal	dark cuticle pigment over e1 on TM3
3	ebony	pFP573	1	no	internal donor plasmid	internal donor plasmid	vermillion	vermillion	Sense	Out	7bp del	none	dark cuticle pigment	dark cuticle pigment over e1 on TM3
4	ebony	pFP573	2	no	ebony 3' insertion site	ebony 3' insertion site	vermillion	vermilllion	Antisense	N/A	5bp del, 25bp ins	none	dark cuticle pigment	dark cuticle pigment over e1 on TM3
5	ebony	pFP573	3	yes	3' end of donor plasmid	3' end of donor plasmid	vermillion	bad sequence data	Sense	Out	8bp deletion	none	dark cuticle pigment	dark cuticle pigment over e1 on TM3
6	hh	GP01881	1	no	bad sequence data	no bands	vermillion	Band 1: hh 5' insertion site Band 2: hh 3' insertion site Band 3: vermillion	Antisense	N/A	1897bp del, 8bp ins	none	lethal	fail to complement hh[AC] and Df(3R)ED5296
7	Mhc	GP00583	1	no	Mhc 5' insertion site	no bands	vermillion	Band 1: Mhc 5' insertion site Band 2: vermillion	Sense	Out	10bp del, 3bp ins	muscle	lethal	fail to complement P{lacW}Mhck10423 and Df(2L)H20
8	Mhc	GP00583	2	no	Mhc 5' insertion site	no bands	bad sequence data	bad sequence data	Sense	Out	9bp del, 13bp ins	muscle	lethal	fail to complement P{lacW}Mhck10423 and Df(2L)H20
9	Mhc	GP00583	3	no	Mhc 3' insertion site	Mhc 3' insertion site	bad sequence data	bad sequence data	Antisense	N/A	27bp del, 1bp ins	none	lethal	fail to complement P{lacW}Mhck10423 and Df(2L)H20
10	Mhc	GP00583	4	yes	Mhc 3' insertion site	Band 1: 3' end of donor plasmid Band 2: 3' end of donor plasmid	bad sequence data	Band 1: 5' end of donor plasmid Band 2: internal donor plasmid	Sense	Out	2bp del, 4bp ins	none	lethal	fail to complement P{lacW}Mhck10423 and Df(2L)H20
11	Mhc	GP00583	5	yes	Mhc 3' insertion site	Band 1: Mhc 3' insertion site Band 2: 3' end of donor plasmid	5' end of donor plasmid	5' end of donor plasmid	Antisense	N/A	2bp deletion	none	lethal	fail to complement P{lacW}Mhck10423 and Df(2L)H20
12	Myo1a	GP00364	1	no	Myo1a 5' insertion site	Myo1a, 5' insertion site	internal donor plasmid	internal donor plasmid	Sense	Out	4bp del, 2bp ins	gut	viable	N/A
13	esg	GP00225	1	yes	esg, 3' insertion site	Band 1: esg, 3' insertion site Band 2: bad sequence data	vermillion	Band 1: 5' end of donor plasmid Band 2: vermillion	Antisense	N/A	25bp del, 10bp	none	lethal	fail to complement P{enG}esg[G66]
14	FK506-bp2	GP02894	1	no	FK506-bp2 3' insertion site	FK506-bp2 3' insertion site	FK506-bp2 5' insertion site	Band 1: FK506-bp2 5' insertion site Band 2: vermillion	Antisense	N/A	30bp del	none	viable	N/A
15	wg	GP06461	1	yes	wg 3' insertion site	Band 1: wg 3' insertion site Band 2: 3' end of donor plasmid	vermillion	Band 1: wg 5' insertion site Band 2: 5' end of donor plasmid	Sense	In	45bp del, 21bp ins	wingless, strong expression	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291
16	wg	GP06461	2	no	3' end of donor plasmid	3' plasmid	wg 5' insertion site	5' end of donor plasmid	Antisense	N/A	33bp del, 11bp ins	none	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291
17	wg	GP06461	3	yes	wg 5' insertion site	Band 1: wg 5' insertion site Band 2: wg 3' insertion site Band 3: 3' end of donor plasmid	vermillion	wg 3' insertion site	Sense	Out	21bp del, 11bp ins	none	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291
18	wg	GP06461	4	no	wg 5' insertion site	Band 1: wg 5' insertion site Band 2: 3' end of donor plasmid	wg 3' insertion site	Band 1: wg 3' insertion site Band 2: attB sequence?	Sense	Out	32bp del, 7bp ins	wingless, weak expression	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291
19	wg	GP06461	5	no	wg 5' insertion site	wg 5' insertion site	wg 3' insertion site	vermillion	Sense	Out	28bp del, 5bp ins	none	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291
20	wg	GP06461	6	no	wg 5' insertion site	Band 1: wg 5' insertion site Band 2: wg 3' insertion site	vermillion	no band	Sense	In	21bp insertion	none	lethal	fail to complement wg[l-17], wg[l-8], and Df(2L)BSC291

Supplemental Table 4 – Molecular and phenotypic characterization of 20 RFP+ fly strains, each carrying a distinct *CRISPaint-T2A-Gal4* insertion.

gene	sgRNA name	sgRNA sequence	DRSC efficiency score
ebony	pFP545	gTGGCCATCTGGAAGGCTGG	5.56968
ebony	pFP573	gTCTACACCTCGGGCAGTAC	5.08877
wg	GP06461	GGGGCCGGGGCTCCATGTGG	5.61857
FK506-bp2	GP02894	TGTAGTGGACCGTGACCTTT	6.5446
alphaTub84B	GP05054	GCTGACGGTAGGTTCCGGTA	6.20359
esg	GP00225	CTCCACCAACATGTCTTCCA	9.35069
Myo1a	GP00364	ATGGCTATGCAACGGGAAGC	7.98579
btl	GP00400	GGCAAAAGTGCCGATCACGC	3.52487
Mhc	GP00583	CTGAAGCCCCGCATCAAGGT	8.00559
hh	GP01881	TAACCACAGCTCAGTGCCTT	6.99912
Desat1	GP03252	AAGCTGCAGGAGGACTCCAC	6.60024
ap	GP05302	CACCACCTGTAGCACATCAA	5.73411

Supplemental Table 5 – Efficiency scores for 12 sgRNAs used in germ line knock-ins

Name	Sequence	How used
JB880_frame+0_gRNA_top	GTCGgccagtacccaaaaagcggg	for cloning pCFD3-CRISPaint_frameselector
JB881_frame+0_gRNA_bot	AAACcccgtttttgggtactggc	for cloning pCFD3-CRISPaint_frameselector
JB882_frame+1_gRNA_top	GTCGggccagtacccaaaaagcgg	for cloning pCFD3-CRISPaint_frameselector
JB883_frame+1_gRNA_bot	AAACcccgtttttgggtactggcc	for cloning pCFD3-CRISPaint_frameselector
JB884_frame+2_gRNA_top	GTCGgggccagtacccaaaaagcg	for cloning pCFD3-CRISPaint_frameselector
JB885_frame+2_gRNA_bot	AAACcgtttttgggtactggccc	for cloning pCFD3-CRISPaint_frameselector
JB886_target-T2A-Gal4overlap_gBlock	ttcacgacctgaggcgcgccgggccagtacccaaaaagcgggggggtccggtggaagcggaggtagcgcggccgccgagggccgcggcagcctgctgacctgcggcgatgtggaggagaaccccgggcccGCTAGCatgaagctactgtcttctatcgaacaagcatgcgatatttgccgacttaaaaagctcaagtgctccaaagaaaaccgaagtgcgccaagtgtct	gBlock containing CRISPaint site and T2A and overlap sequence
JB877_Gal4_F	atgaagctactgtcttctatcgaaca	to amplify Gal4-3xP3RFP
JB878_target-T2A-Gal4_P3RFP_R	gggaacaaaagctggagctcataacttcgtatagcatacattatacgaagttatCGTATGGGCCTTCGCTGCTTACAG	to amplify Gal4-3xP3RFP
JB915_sfGFP_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcGTGTCCAAGGGCGAGGAG	for cloning into JAB290 cut with NheI/KpnI
JB916_sfGFP_HIKI_R	caaagatcctctagaggtaccCTACTTGTACAGCTCATCCATGC	for cloning into JAB290 cut with NheI/KpnI
JB1000_LexGAD_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGCCACCCAAGAAGAAGC	for cloning into JAB290 cut with NheI/KpnI
JB1001_LexGAD_HIKI_R	caaagatcctctagaggtaccCTACTCCTTCTTTGGGTTCGG	for cloning into JAB290 cut with NheI/KpnI
JB998_QF2_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGCCACCCAAGCGCAAA	for cloning into JAB290 cut with NheI/KpnI
JB999_QF2_HIKI_R	caaagatcctctagaggtaccTCACTGTTCGTATGTATTAATGTCG	for cloning into JAB290 cut with NheI/KpnI
JB1055_Cas9-T2A-EGFP_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGGATTACAAGGATCACGATG	for cloning into JAB290 cut with NheI/KpnI
JB1056_Cas9-T2A-EGFP_HIKI_R	caaagatcctctagaggtaccTTAGTACAGCTCGTCCATGCC	for cloning into JAB290 cut with NheI/KpnI
JB1002_FLPo_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcatgagccagttcgacatcct	for cloning into JAB290 cut with NheI/KpnI
JB1003_FLPo_HIKI_R	caaagatcctctagaggtacctcagatccgcctgttgatgtagc	for cloning into JAB290 cut with NheI/KpnI
JB1004_Gal80_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGGACTACAACAAGAGATCTTCG	for cloning into JAB290 cut with NheI/KpnI
JB1005_Gal80_HIKI_R	caaagatcctctagaggtaccTTATAAACTATAATGCGAGATATTGCTAA	for cloning into JAB290 cut with NheI/KpnI
JB1008_Nluc_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGGTCTTCACACTCGAAGATTT	for cloning into JAB290 cut with NheI/KpnI
JB1009_Nluc_HIKI_R	caaagatcctctagaggtaccTTACGCCAGAATGCGTTC	for cloning into JAB290 cut with NheI/KpnI
JB1032_Gal4DBD_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGCTGGAGATCCGCGCC	for cloning into JAB290 cut with NheI/KpnI
JB1033_Gal4DBD_HIKI_R	caaagatcctctagaggtaccTTACGATACCGTCAGTTGCC	for cloning into JAB290 cut with NheI/KpnI
JB1030_p65_HIKI_T2A_F	tgtggaggagaaccccgggcccgctagcATGGATAAAGCGGAATTAATTCC	for cloning into JAB290 cut with NheI/KpnI
JB1031_p65_HIKI_R	caaagatcctctagaggtaccTTACTTGCCGCCGCCCAG	for cloning into JAB290 cut with NheI/KpnI
JB917_sfGFP_HIKI_F	ggtggaagcggaggtagcgcggccgccGTGTCCAAGGGCGAGGAG	for cloning into JAB290 cut with NotI/KpnI
JB916_sfGFP_HIKI_R	caaagatcctctagaggtaccCTACTTGTACAGCTCATCCATGC	for cloning into JAB290 cut with NotI/KpnI
JB1355_FK506-bp2_geno_1F	acgcgccaaaatacaaaaac	amplification of endogenous target gene and knock-in
JB1356_FK506-bp2_geno_1R	GCCTATTCGACCTTGAGCAG	amplification of endogenous target gene
JB1357_alphaTub84B_geno_1F	tttgtgtgggcaaaattcaa	amplification of endogenous target gene and knock-in
JB1358_alphaTub84B_geno_1R	GCTTGGACTTCTTGCCGTAG	amplification of endogenous target gene
JB1359_esg_geno_1F	CGTTTGGTATTTGTGCATCG	amplification of endogenous target gene and knock-in
JB1360_esg_geno_1R	GTAGGGCGACATGTGGAAGT	amplification of endogenous target gene
JB1361_btl_geno_1F	aactaagggaggggcaaaaa	amplification of endogenous target gene and knock-in
JB1362_btl_geno_1R	CGTCCACCAAGGATTTGAGT	amplification of endogenous target gene
JB1363_Desat1_geno_1F	aatccacctggtgcttgttc	amplification of endogenous target gene and knock-in
JB1364_Desat1_geno_1R	GTAACCGAAGGCGATGATGT	amplification of endogenous target gene
JB1365_ap_geno_1F	ttgcaaatctgtcaggaacg	amplification of endogenous target gene and knock-in
JB1366_ap_geno_1R	ATCTGGACACGAGGATGAGG	amplification of endogenous target gene
JB959_ebony_geno_F	gcattagcctgcattgcata	amplification of endogenous target gene and knock-in
JB960_ebony_geno_R	CACGCCCTCATCGAAATAGT	amplification of endogenous target gene
JB961_wg_geno_F	CAGTTAAGCGTTGGCACTGA	amplification of endogenous target gene and knock-in
JB962_wg_geno_R	ttgttgcatctctgcggtag	amplification of endogenous target gene
JB963_Myo1a_geno_F	TCGTCGTCATCAACAGAAGC	amplification of endogenous target gene and knock-in
JB964_Myo1a_geno_R	tctggagtggaaccgaaaac	amplification of endogenous target gene
JB965_Mhc_geno_F	cggctaaagactgacccaaa	amplification of endogenous target gene and knock-in
JB966_Mhc_geno_R	CTCTTGCTCCATGACGAACA	amplification of endogenous target gene
JB967_hh_geno_F	TCGTACTCGCACTCGAACAC	amplification of endogenous target gene and knock-in
JB1388_hh_geno_6F	aaatcaaagctggaccaaatc	amplification of endogenous target gene and knock-in, used with hh #1 insertion
JB968_hh_geno_R	GTTGTAGTTGGGCACGAGGT	amplification of endogenous target gene
JB900_T2A_R	cggggttctcctccacat	reverse primer for amplifying T2A-Gal4 insert in sense orientation
JB958_T2A-Gal4_3'_F	gttttcccagtcacgacgtt	reverse primer for amplifying T2A-Gal4 insert in antisense orientation
JB659_3P3dsred_seq1F	ACTCCAAGCTGGACATCACC	to amplify 3xP3-dsred as a control
JB660_3P3dsred_seq1R	CGAGGGTTCGAAATCGATAA	to amplify 3xP3-dsred as a control
JB713_Rp49_F	ATCGGTTACGGATCGAACAA	to amplify endogenous Rp49 gene as a control
JB714_Rp49_R	GACAATCTCCTTGCGCTTCT	to amplify endogenous Rp49 gene as a control
JB1051_alphaTub84B_C-term_F	CCTTCGTCCACTGGTACGTT	amplification of endogenous target gene and knock-in
JB1052_Act5c_C-term_F	CGTCGACCATGAAGATCAAG	amplification of endogenous target gene and knock-in
JB1053_His2Av_C-term_F	CTCCTCGCCACTTACAGCTC	amplification of endogenous target gene and knock-in
JB1054_Lam_C-term_F	GCCGACAACACTAGGACGAT	amplification of endogenous target gene and knock-in
JB1050_mNeonGreen_R	GGGAGAGAGGCGTTATCCTC	reverse primer for amplifying gene-mNeonGreen insert
JB1120_act5c_seqnextgenR	CGACTTCTCCTCCTCCTCT	amplification of endogenous target gene
JB1121_His2av_seqnextgenR	TCGTCGGTGTTTTAGCTTGTC	amplification of endogenous target gene
JB1122_alphatub_seqnexgenR	GCGATTGGAAGCGTAAACAC	amplification of endogenous target gene
JB1123_Lam_seqnextgenR	GTGTTGTGCTGCGTTTGATT	amplification of endogenous target gene
JB1192_Lam_2F	GCGGCTAATCAACGAGAAAG	amplification of non-knock-in Lamin gene
JB1193_Lam_2R	TCTGTTGTCAGGAGCGTTTG	amplification of non-knock-in Lamin gene
JB1194_Lam_3F	ACGAGGAGCAGATGCAGATT	amplification of non-knock-in Lamin gene
JB1195_Lam_3R	GGTCTAAACCGGGAGAAAGC	amplification of non-knock-in Lamin gene
JB1196_Lam_4F	AGCTGCAGAACCTGAACGAT	amplification of non-knock-in Lamin gene
JB1197_Lam_4R	ACTAGCCGAACCCAGGATTT	amplification of non-knock-in Lamin gene
JB1198_Lam_5F	cccattacaagcgacgattt	amplification of non-knock-in Lamin gene
JB1199_Lam_5R	GAACAGCTCCACTCCTCCAG	amplification of non-knock-in Lamin gene
JB1200_Lam_6F	GTCTCGGTCTGCTCCTCATC	amplification of non-knock-in Lamin gene
JB1201_Lam_6R	GAATGGCATAGCCACCACTT	amplification of non-knock-in Lamin gene
JB1202_Lam_7F	CATTTGCAAGATGGTGGTTG	amplification of non-knock-in Lamin gene
JB1203_Lam_7R	ccaattaggccaacactgct	amplification of non-knock-in Lamin gene
JB1050_mNeonGreen_R	GGGAGAGAGGCGTTATCCTC	amplification and sequencing primer, 5' end of mNeonGreen
JB1423_Not1-MCS-BamHI_gBlock	agggtttcccagtcacgacgttgtaaaacgacggccagtgaattgtaatacgactcactatagggcgaattgggtacaagcttcacgacctgaggcgcgccgggccagtacccaaaaagcgggggggtccggtggaagcggaggtagcgcggccgcacGCTAGCtgCTGAGacGGTACCtaGAATTCacAGATCTataacttcgtataatgtatgctatacgaagttatcgtacgGGATCTAATTCAATTAGAGACTAATTCAATTAGAGCTAATTCAATTAGGATCCAAGCTTATCGATTTCGAACCCTCGACCGCCGGAGTATAAATAGAGGCGCTTCGTCTACGGAGCGACAATTCAATTCAAACAA	gBlock containing multiple cloning site to construct pCRISPaint-3xP3-RFP
JB1426_pCRISPaint_3xP3RFP_backbone_F	gggccgcgactctagatcataatc	to amplify pCRISPaint-3xP3-RFP backbone to swap out RFP sequence
JB1427_pCRISPaint_3xP3RFP_backbone_R	ggtggcgaccggcttcga	to amplify pCRISPaint-3xP3-RFP backbone to swap out RFP sequence
JB1428_GFP_overlapCRISPaint3xP3RFP_F	gctcgaagccggtcgccaccatggtgtccaagggcgag	to amplify EGFP to Gibson clone into pCRISPaint-3xP3-RFP
JB1429_GFP_overlapCRISPaint3xP3RFP_R	atgatctagagtcgcggcccctacttgtacagctcatccatgc	to amplify EGFP to Gibson clone into pCRISPaint-3xP3-RFP
JB939_PCR1fwrd_frame0_1_2_pCFD5	GCGGCCCGGGTTCGATTCCCGGCCGATGCAgccagtacccaaaaagcgggGTTTTAGAGCTAGAAATAGCAAG	to construct pCFD5-frame_selector_0,1,2
JB940_PCR1rev_frame0_1_2_pCFD5	ccgcttttgggtactggccTGCACCAGCCGGGAATCGAACCC	to construct pCFD5-frame_selector_0,1,2
JB941_PCR2fwrd_frame0_1_2_pCFD5	ggccagtacccaaaaagcggGTTTTAGAGCTAGAAATAGCAAG	to construct pCFD5-frame_selector_0,1,2
JB942_PCR2rev_frame0_1_2_pCFD5	ATTTTAACTTGCTATTTCTAGCTCTAAAACcgcttttgggtactggcccTGCACCAGCCGGGAATCGAACCC	to construct pCFD5-frame_selector_0,1,2
JB1294_ebony_LHA_T2A-Gal4_2F	tgggtacaagcttcacgacctgagggcacgggtaattgtcgttctt	to amplify ebony left homology arm
JB1295_ebony_LHA_T2A-Gal4_2R	ccccgctttttgggtactggcccggtGCCTTCCAGATGGCCAGC	to amplify ebony left homology arm
JB975_ebony_RHA_T2A-Gal4_F	atgtatgctatacgaagttatgagctTGGCGGCGCGTATTTGCC	to amplify ebony right homology arm
JB976_ebony_RHA_T2A-Gal4_R	accctcactaaagggaacaaaagctggagctCCTCAGGAACCTGCGAGTAG	to amplify ebony right homology arm
JB1484_ebony_HDRgeno_F	gggcaaacatagtgcaggtt	use with to verify ebony-T2A-Gal4 HDR knock-in
JB1485_ebony_HDRgeno_R	AGACCCTTGGGCAGGTAGTT	to verify ebony-T2A-Gal4 HDR knock-in
JB1045_SPLNK-GATC-TOP	GATCCCACTAGTGTCGACACCAGTCTCTAATTTTTTTTTTCAAAAAAA	splinkerette oligos for annealing
JB1046_SPLNK-BOT	CGAAGAGTAACCGTTGCTAGGAGAGACCGTGGCTGAATGAGACTGGTGTCGACACTAGTGG	splinkerette oligos for annealing
JB1047_SPLNK#1	CGAAGAGTAACCGTTGCTAGGAGAGACC	splinkerette round 1 primer
JB1048_SPLNK#2	GTGGCTGAATGAGACTGGTGTCGAC	splinkerette round 2 primer
JB1057_T2A-Gal4_5'#1	agcggagacctttggtttt	5' splinkerette round 1 primer
JB1058_T2A-Gal4_5'#2	tcggtttttctttggagcac	5' splinkerette round 2 primer
JB1059_T2A-Gal4_5'Seq	cggggttctcctccacat	5' splinkerette sequencing primer
JB1060_T2A-Gal4_3'#1	gtgctgcaaggcgattaagt	3' splinkerette round 1 primer
JB1061_T2A-Gal4_3'#2	gttttcccagtcacgacgtt	3' splinkerette round 2 primer
JB1062_T2A-Gal4_3'Seq	GTAAAACGACGGCCAG	3' splinkerette sequencing primer

Supplemental Table 7 – Oligo and dsDNA sequences