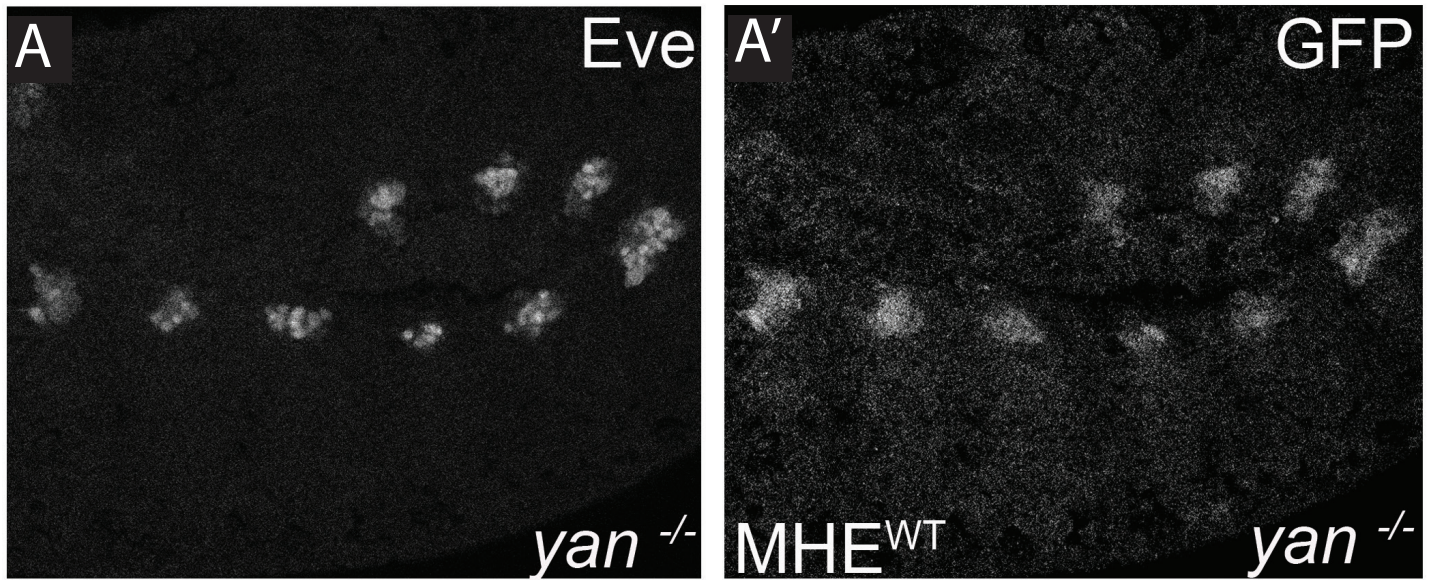
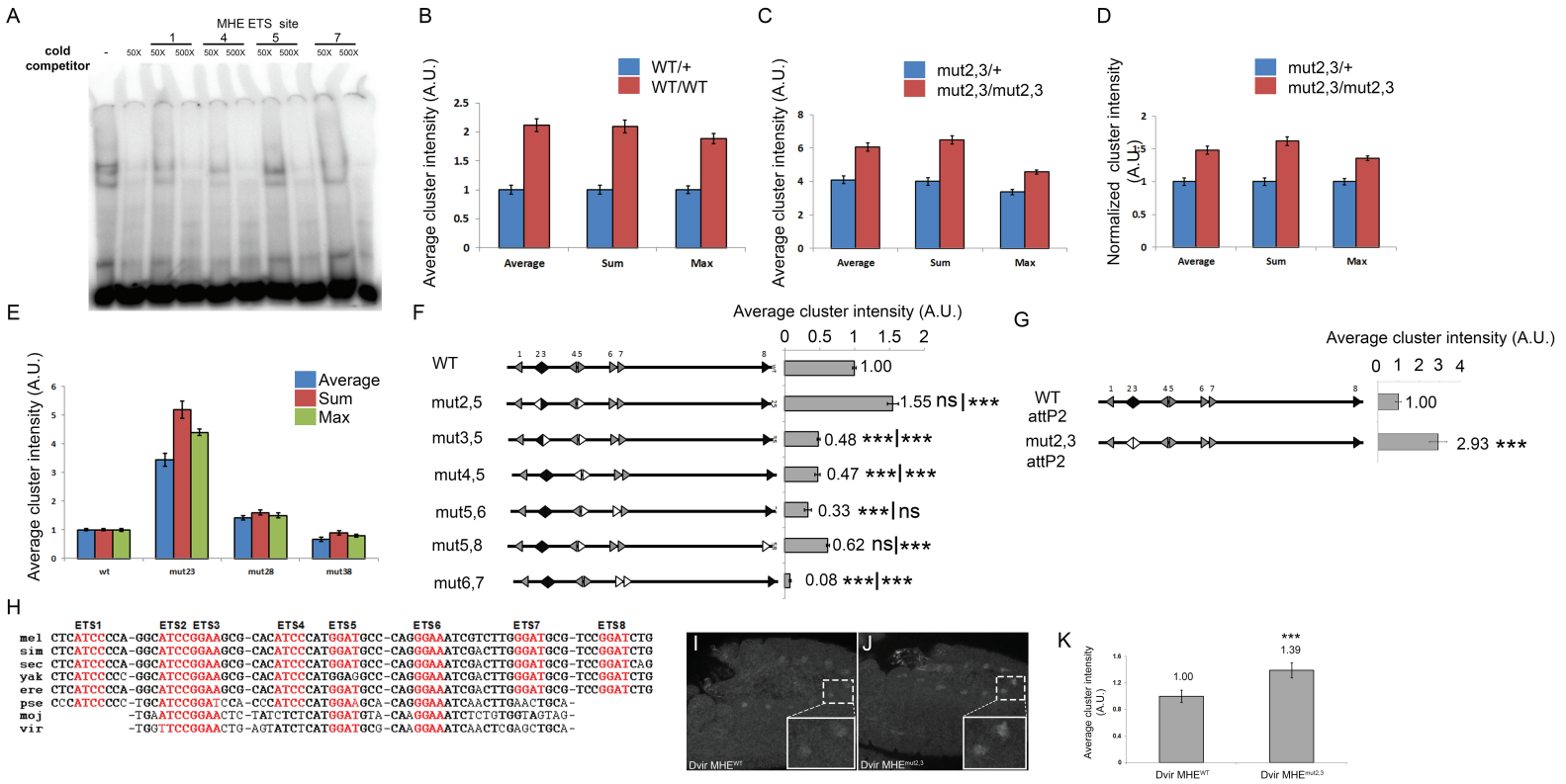


Boisclair Lachance_Supplemental Fig. S1



Boisclair Lachance_Supplemental Fig. S2



L

D. mel>MHE

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AC**ATCC**CAT**GGAT**GCCATCAATTAGCATACAATTAATAAATGCTTAAACAG**GGAA**ATCGACTT**GGAT**GCGAGTGGTTCG
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D. sim>MHE

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D. sec>MHE

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D. yak>MHE

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D. ere>MHE

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CTCGATGGG**CATCCGGAA**CGGG**GGAA**GCCAAAAATATACACAC**ATCC**CAT**GGAT**GCCATCAATTAGCATACAATTA
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GTGGCGGGCAGCAGATCAAAGCGACGACAACATAATTGCTGCTTCACTT**CAC**AGT**TCT**CAGGC**ACT**TAAGATATGATATG
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D. pse>MHE

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ATCTGGGACTGTAAGTGGCAACGGCTGCGGCTGGGAGCAGATCAAAGCGACGACAACATAATTGCTGCTTCACTT**CAC**AG
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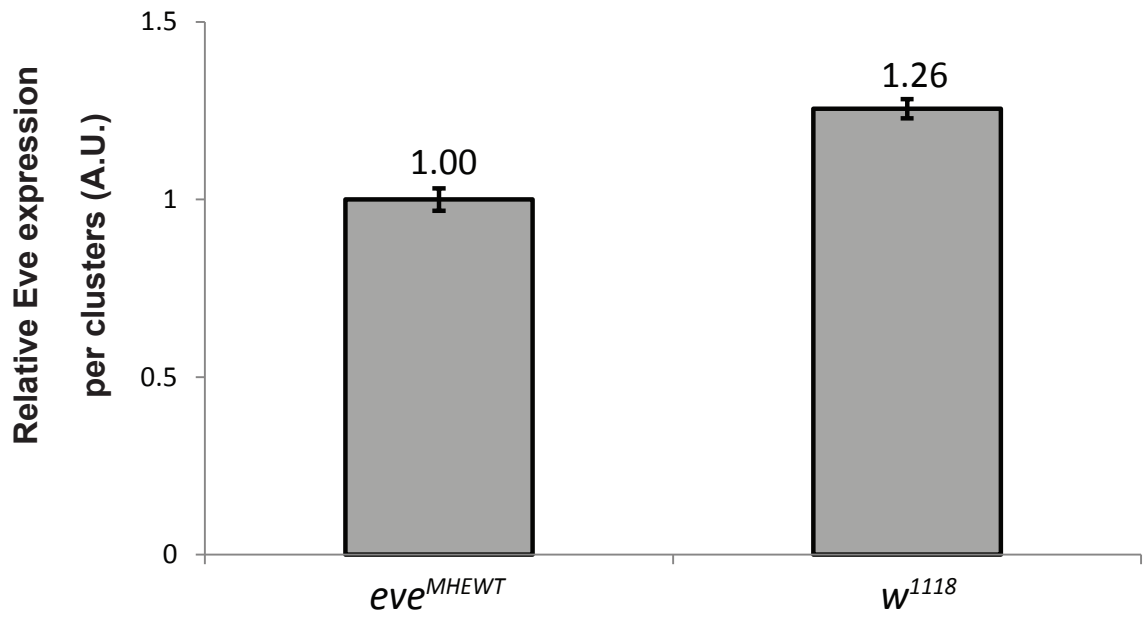
D. moj>MHE

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TGTGAGAA

D. vir>MHE

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A



Boisclair Lachance_Supplemental Fig. S4

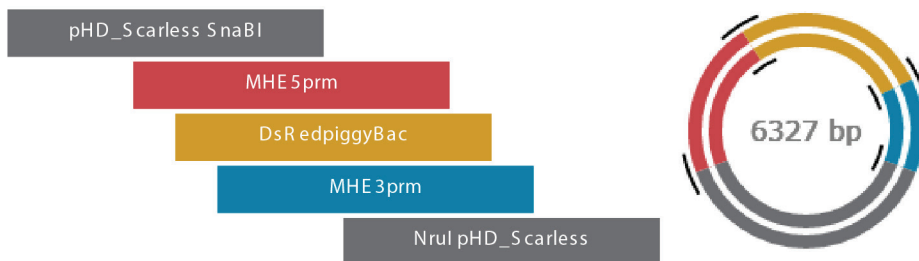
A

Product Version	E 5520 - NEBuilder High-Fidelity DNA Assembly Cloning Kit
No. of Fragments	2-3 fragments (including vector)
Construct Length	less than 10 Kb
Min. Overlap	25 bp
PCR Polymerase	Q5 High-Fidelity DNA Polymerase
PCR Primer Conc.	500 nM
Min. Primer Length	19 nt

Vector Digestion

Vector backbone digested with SnaBI and NruI

Fragment Arrangement



Required Primers

Overlaps	Oligo (Uppercase = gene-specific primer)	Anneals	F/R	3' T _m	3' T _a *	6-Frame
pHD_Scarless	agtatttattatgcatttagaatacGAAGCGGAACAGAATGCCA	MHE 5prm	Fwd	63.5°C	62.9°C	view
DsRedpiggyBac	tttctagggttaaTTAAGGACAACCTCTCAAGTG	MHE 5prm	Rev	59.9°C	62.9°C	view
MHE 5prm	agttgtcctttaTTAACCTAGAAAGATAATCATATTG	DsRedpiggyBac	Fwd	55.5°C	58.5°C	view
MHE 3prm	tacccattaaaggtaccTTAACCTAGAAAGATAGTCTG	DsRedpiggyBac	Rev	55.8°C	58.5°C	view
DsRedpiggyBac	tttctagggttaaaggtaccTTAATGGTGAGGCGCGGA	MHE 3prm	Fwd	67.4°C	66.6°C	view
pHD_Scarless	aattaaccaattctgaacattatcgCTGACAATTAGCCCTGAACC	MHE 3prm	Rev	63.6°C	66.6°C	view

Boisclair Lachance_Supplemental Fig. S4

B

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pHD-Scarless

MHE-5prm
PCR AB

DsRedpiggyBac
PCR EF

MHE-3prm
PCR CD

pHD-Scarless

Contains Amp^R gene and origin of replication of pHD-Scarless (all sequence between SnaB1 and NruI)

Guide RNA site 1
GCC upstream changed to TGC introducing a FspI restriction site

ETS site 2 and 3 mutated to GGCTACCGGCTAG in eve^{MHEmut2,3}

Contains the eye marker for identifying successful homologous directed repair events and piggyBac transposable element arms for piggyBac mediated excision of this cassette

Guide RNA site 1
CGG downstream changed to CCC introducing a BamH1 restriction site

Contains Amp^R gene and origin of replication of pHD-Scarless (all sequence between SnaB1 and NruI)

Boisclair Lachance_Supplementary Table 1

TABLE S1: Mendelian ratio of *eve*^{MHEWT} or *eve*^{MHEmut23} in the different genotypes shown in Figure 3.

Genotype	actual	expected
<i>eve</i> ^{MHEWT} /CTG	71	77
<i>eve</i> ^{MHEWT}	45	39
Total	116	116
<i>eve</i> ^{MHEmut23} /CTG	457	461
<i>eve</i> ^{MHEmut23}	235	231
Total	692	692
<i>Pnt-GFP, eve</i> ^{MHEWT} /CTG	453	481
<i>Pnt-GFP, eve</i> ^{MHEWT}	268	240
Total	721	721
<i>Pnt-GFP, eve</i> ^{MHEmut23} /CTG	1067	1006
<i>Pnt-GFP, eve</i> ^{MHEmut23}	442*	503
Total	1509	1509
<i>Pnt-GFP, eve</i> ^{MHEWT} /CTG or <i>yan</i> ^{E443} , <i>eve</i> ^{MHEWT} /CTG	338	367
<i>Pnt-GFP, eve</i> ^{MHEWT} / <i>yan</i> ^{E443} , <i>eve</i> ^{MHEWT}	213	184
Total	551	551
<i>Pnt-GFP, eve</i> ^{MHEmut23} /CTG or <i>yan</i> ^{E443} , <i>eve</i> ^{MHEmut23} /CTG	502	499
<i>Pnt-GFP, eve</i> ^{MHEmut23} / <i>yan</i> ^{ER443} , <i>eve</i> ^{MHEmut23}	246	249
Total	748	748

* This lower survival of *Pnt-GFP, eve*^{MHEmut23} is found significant by chi-square analysis (p=0.000865)