Boisclair Lachance_Supplemental Fig. S2

D. mel>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. sim>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. sec>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. yak>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. ere>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. pse>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. moj>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC

D. vir>MHE

GGATCCGTCCTCCACTATTTCCATTTGCGGTCTGGGAAGTTGGCGATGGTTGGGC
ATCCGGAGGCGGGGGCAGCCAAAAATATACCC
### Product Version
E5520 - 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

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**Vector Digestion**

Vector backbone digested with SnaB1 and NruI

**Fragment Arrangement**

![Fragment Arrangement Diagram](image)

**6327 bp**

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**Required Primers**

| Overlaps       | Oligo (Uppercase = gene-specific primer)                                                                 | Anneals    | F/R | 3’ Tm | 3’ Ta | 6-Frame |
|----------------|--------------------------------------------------------------------------------------------------------------|------------|-----|-------|-------|---------|
| pHD_Scarless   | aattatatggttagattgattatgcatttgaatatcGAAGCGGAACAGAATGCCA                                                    | MHE 5’rm   | Fwd | 63.5°C| 62.9°C| view    |
| DsRedpiggyBac  | tttctaggttaAAAGGACAACTGCCAAGTG                                                      | MHE 5’rm   | Rev | 59.9°C| 62.9°C| view    |
| MHE 5’rm       | agtatgctattaatATTTACCCCTAGAAGGATAATCATATTG                                                                | DsRedpiggyBac| Fwd | 55.5°C| 58.5°C| view    |
| MHE 3’rm       | tacccctagggttagttacTAAAGGACAACTGCCAAGTG                                                           | DsRedpiggyBac| Rev | 55.8°C| 58.5°C| view    |
| DsRedpiggyBac  | tttctaggttaaaAAAGGACAACTGCCAAGTG                                                                | MHE 3’rm   | Fwd | 67.4°C| 66.6°C| view    |
| pHD_Scarless   | aattaaccaattctgccattatcggCTGACAGTAGGCCTGACGACC                                                      | MHE 3’rm   | Rev | 63.6°C| 66.6°C| view    |
### TABLE S1: Mendelian ratio of $eve^{MH_{\text{HEWT}}}$ or $eve^{MH_{\text{HEmut23}}}$ in the different genotypes shown in Figure 3.

| Genotype                                      | actual | expected |
|-----------------------------------------------|--------|----------|
| $eve^{MH_{\text{HEWT}}}/CTG$                  | 71     | 77       |
| $eve^{MH_{\text{HEWT}}}$                      | 45     | 39       |
| Total                                         | 116    | 116      |
| $eve^{MH_{\text{HEmut23}}}/CTG$               | 457    | 461      |
| $eve^{MH_{\text{HEmut23}}}$                   | 235    | 231      |
| Total                                         | 692    | 692      |
| $Pnt$-GFP, $eve^{MH_{\text{HEWT}}}/CTG$       | 453    | 481      |
| $Pnt$-GFP, $eve^{MH_{\text{HEWT}}}$           | 268    | 240      |
| Total                                         | 721    | 721      |
| $Pnt$-GFP, $eve^{MH_{\text{HEmut23}}}/CTG$   | 1067   | 1006     |
| $Pnt$-GFP, $eve^{MH_{\text{HEmut23}}}$        | 442*   | 503      |
| Total                                         | 1509   | 1509     |
| $Pnt$-GFP, $eve^{MH_{\text{HEWT}}}/CTG \text{ or } yan^{E443}$, $eve^{MH_{\text{HEWT}}}/CTG$ | 338    | 367      |
| $Pnt$-GFP, $eve^{MH_{\text{HEWT}}}/ yan^{E443}$, $eve^{MH_{\text{HEWT}}}$ | 213    | 184      |
| Total                                         | 551    | 551      |
| $Pnt$-GFP, $eve^{MH_{\text{HEmut23}}}/CTG \text{ or } yan^{E443}$, $eve^{MH_{\text{HEmut23}}}/CTG$ | 502    | 499      |
| $Pnt$-GFP, $eve^{MH_{\text{HEmut23}}}/ yan^{ER443}$, $eve^{MH_{\text{HEmut23}}}$ | 246    | 249      |
| Total                                         | 748    | 748      |

* This lower survival of $Pnt$-GFP, $eve^{MH_{\text{HEmut23}}}$ is found significant by chi-square analysis (p=0.000865)