

Supplementary Online Material:

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                Genomic Sequence                                P element insertion sequence (X06779 position 3907-3986 reverse complement)
...CCCGATTTTGACCCCTCTTGCTCCTCTTTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCACTTAACGTATGCTTGAATAAGTGCAGTGAAGG...
RNA Seq reads that cross over into the P element:
CCCGATTTTGACCCCTCTTGCTCCTCTTTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGT
ATTTTGACACTCTTGCTCCTCTTTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCC
CTTGCTCCTCTTTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAG
CTTGCTCCTCTTTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAG
TTTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCAC
TTCAGTTGTATTTCCCGAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCACT
CCAGCCGTGGACACAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCAAATTAACGTATGCTTGCA
CACAAAGTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCACTTAACGTATGCTTGAATAAGTGCAG
GTGAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCACTTAACGTATGCTTGAATAAGTGCAGTGAAG
GAAACAACATGCCATGTAC|CATGATGAAATAACATAAGGTGGTCCCGTCGGCAAGAGACATCCACTTAACGTATGCTTGAATAAGTGCAGTGAAGG

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RNA Seq reads from the *heph*⁰³⁴²⁹ mutant that cross over into the P element. The genomic reference sequence shown on the upper left is within the *heph* gene on chromosome 3R from 27756335 to 27756257 (reverse complement). The P element insertion sequence on the upper right was taken from a section of the pPI25.1 transformation vector (Genbank accession #X06779). Shown below the reference sequences are 100-mer reads that span from the *heph* intronic sequence into the P element sequence. No wild-type (yw) control RNA Seq reads were found with the P element insertion sequence (based upon checking 20 nt sections of the P element insertion sequence). There did not appear to be any other P element insertion site in the *heph*⁰³⁴²⁹ sample based on RNA Seq reads. No reads were found that spanned the other end of the P element insertion into the *heph* gene.

Supplementary Table 1. Primers used for *heph*⁰³⁴²⁹ validation of differential expression

Gene (isoform)	Forward primer	Reverse primer
Z600	GCAAATCAGCAACGTCAAGC	TTTGCGAGCCTTGATGAAAT
CanA1	TGCTAGTCAACATCCTGAACATTTGCTC	CCGAATCTTATCCGAATTATCTCTTTG
LpR2	GCCACTGTTCTCATCTTTGCCTGC	CTATTTGACGTAATCATTCTG
CG11309	GCTCGATGCTTTACCTGTTTGTGGGC	GTTAAGTCGCCTGCATGCCGATAACGCT
Hrg, P1	GATACACTCTCCTGCCAAAAGTTC	TATTTAAGTTTGCTCTCCAAGAAG
Hrg, P2	TGTCAATTAAGTGTGGTACAC	TATTTAAGTTTGCTCTCCAAGAAG
Bsg25D, P1	AAGTCCCTGCTGAAGCTCTGCTCAC	GCAACTGGTCCCTCGTCCGTATTGTCCG
Bsg25D, P2	AGTTAACAGATCCGATAATTGAGAAGCTAGC	GCAACTGGTCCCTCGTCCGTATTGTCCG
zerknüllt	CCAAAGTCAGGTCCCCACGA	GGAGGTTACGCACGGTTCA
twisted gastrulation	GCCAGTAGCTATCGATGGTTCCAC	ATCGCTCCAGATCCTGTTCCAGCCT
Screw	CCACGCCATCGTCCAAACCCTGAT	ATCTAATGACATCCGCACTCCTTG
mummy	ATTTGTTACCTATTGTGTCCGTTG	AATATTCATATCATTACATGTTCT
BobA	GATGACGAGAACACAAGACTGACT	TACTAACTTAGACTACATTAAAGC
Rumi	ATGAAGGACATCAAGTGCTATTGG	CATGGAATGCGGACAGAGAGATTC
m4	ACGAGCGTCTCTCCAGTCCTGCG	TGGCGTTGGAGGTGCTGCAGTAGA
Cpr49Ad	GCTCCTATTCTACAACATGAGA	GTGTTTTGTATAGACTACATTGGC
Acp1	AACTTTAGACCCCTCAACACGTTA	TTCTTTATTGGCATTATGTAGATG
Zerknüllt	CATCGGTCTGCCTCCCAACT	GGAGGTTACGCACGGTTCA