

Table S1. Primers for amplification of potential promoters of endogenous genes in DBM.

potential promoters	location	Actual length/length in the genome sequence file	Forward primer (5'→3')	Reverse primer (5' →3')
515-1	Upstream of Px02C00515	1131/1131	TTCAAGTAAGAATAACCAAATACATACCCCTCT	TGCCTAGTTCCTATGCGTGAT
515-2	Downstream of Px02C00515	399/3000	GGGTGTGGTGC GGTTCCCTTG	ATTCCCACGTGGT GATCCGTAAAAG
303	Upstream of Px05C00303	877/1008	TTCGCACACTTGTATCACTTCAC	TATAAATAAGAAATAAATGTGAGAGGT CATCAAAT
656-1	Upstream of Px07C00656	676/640	CCAAACAGACTGTAAAAACTGTG	ATTATAATT TATTAGGTACTGATT TACTGTTATT
656-2	Downstream of Px07C00656	417/1316	TTGGATTGTTCTAATAGCTCGCTAC	TCATTTAGCCCTAAGATTTTACTC
217	Upstream of Px18C00217	1702/1702	ATTTATTTTTAATTAGGATAATTAATTAGTTTTAT TCCTG	CTTGATCTGTAACAATGGAATAAAATGTTAAT
336-1	Upstream of Px18C00336	1600/1600	ATGTTAAATACCGCGTCAGGAAC	ATACAACGTTGAATCACATTACCAC
336-2	Downstream of Px18C00336	1607/1607	CCACCTCGCAGACGCAGC	CTTCGAGCCGCAACACAC

Table S2. Primers for amplification of the 217 promoter.

Truncated promoter	The truncated length/Total length	Forward primer (5'→3')	Reverse primer (5'→3')
217-1	1462/1702	ATTGGTAAATACCCAGTCTGAGTG	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-2	1267/1702	CGGTACCTATCTCTCAAGGCAC	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-3	1079/1702	CTGGCTGTACTATGTA CTCGTG	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-4	850/1702	AACGCATCGCATAGTAAACTCAC	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-5	671/1702	GAGAACTGCGCAGTGCAG	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-6	477/1702	CTCTACAATAGGTGTGGCAAGG	CTTGATCTGTAACAATGGAATAAAATGTTAAT
217-7	263/1702	ATGTTCA GTGTACGTTCTTAGG	CTTGATCTGTAACAATGGAATAAAATGTTAAT

Table S3. Primers for construction of pGL3 plasmid with endogenous promoters.

Primer	Sequence (5'→3')
pGL3-Basic-reverse amplification-F	TTGGCATTCCGGTACTGTTGGT
pGL3-Basic-reverse amplification-R	ACTTAGATCGCAGATCTCGAG
Homology arm added to endogenous promoter forward primer	ATCTGCGATCTAAGT
Homology arm added to endogenous promoter reverse primer	GTACCGGAATGCCAA

Table S4. Primers for construction of pB-EGFP plasmid with different promoters.

Primer	Sequence (5'→3')
pB-EGFP-reverse amplification-F1	ATGGTGAGCAAGGGCGAGGAG
pB-EGFP-reverse amplification-R2	AGATCTTAATACGACTCACTATAAGGC
Homology arm added to endogenous promoter forward primer	TGAGTCGTATTAAGATCT
Homology arm added to endogenous promoter reverse primer	TCGCCCTTGCTCACCAT

Table S5. Primers for construction of pB-Neo-EGFP plasmid.

Primer	Sequence (5'→3')
Neo-F	ATGATTGAACAAGATGGATTGCACGCAG
Neo-R(HA)	<u>ACCGCATGTTAGAACAGACTCCTCTGCCCTCGAAGAACTC</u> GTCAAGAACCGCGATAGAAG
pB-EGFP-reverse amplification-F2(HA)	<u>CTTCTAACATGCGGTGACGTGGAGGAGAATCCGGCCC</u> TATGGTGAGCAAGGGCGAGGAGCTGTT
pB-EGFP-reverse amplification-R2	AGATCTTAATACGACTCACTATAAGGC
IE1-F(HA)	<u>TCTTGTTATAGATATCCGATGTCTTGTATGCGCGCGA</u> CATTTC
IE1-R(HA)	<u>CATCTGTTCAATCATCTGGTTGTTACGATCTGTCGC</u>

The underlined part in the table indicates the homology arm added to the primer.

Table S6. Primers for construction of pB-Cas9-Neo plasmid.

Primer	Sequence (5'→3')
pB-Neo-reverse amplification-F	ACGCGAGTTAATTAAGACCCGGGCTGCAGG
pB-Neo-reverse amplification-F	AGATCTTAATACGACTCACTATAAGGGCGAATTGG
Cas9-F(HA)	<u>CCATTGTTACAGATCAAGATGGACAAGAAGTACTC</u> CATTGGGCT
Cas9-R	TCACACCTTCCTCTTCTTCTTGG
217-2-F(HA)	<u>ATAGTGAGTCGTATTAAGATCTCGGTACCTATCTCT</u> CAAGGCAC
217-2-R	CTTGATCTGTAACAATGGAATAAAATGTTAAT
SV40-F(HA)	<u>AGAAGAAGAGGAAGGTGTGAGATCCACCGGATCTA</u> GATAACTG
SV40-R(HA)	TCTTAATTAACTCGCGTTAACGATACAT
pB-Cas9-detection-F	ATGGACAAGAAGTACTCCATTGGGCTC
pB-Cas9-detection-R1	TCACACCTTCCTCTTCTTGGGGTCAGCCC
pB-Cas9-detection-R2	CCACTACGTGAACCATCACCTTAATC

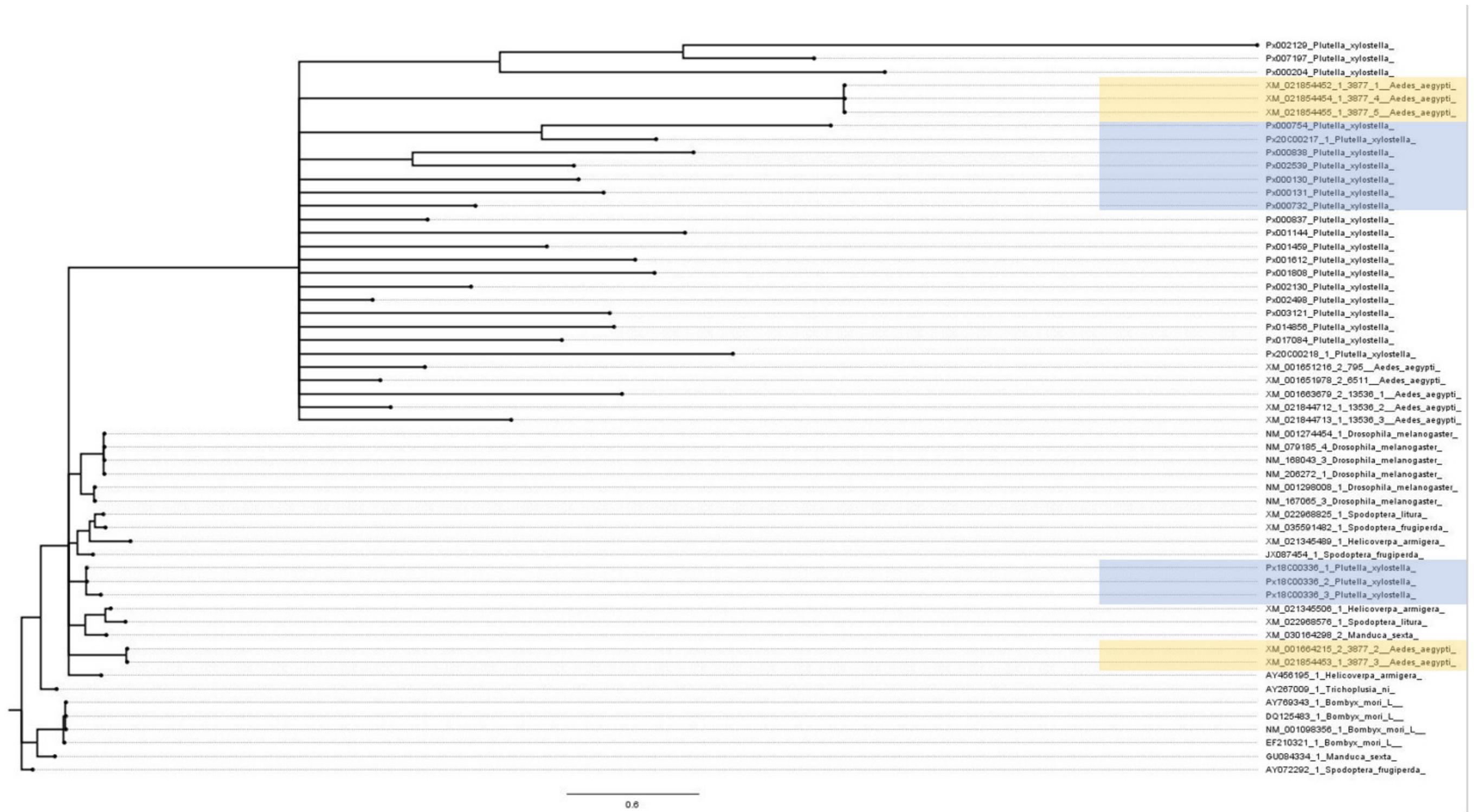
The underlined part in the table indicates the homology arm added to the primer.

Table S7. Primers for construction of pU6-shRNA (EGFP) plasmid and RT-qPCR.

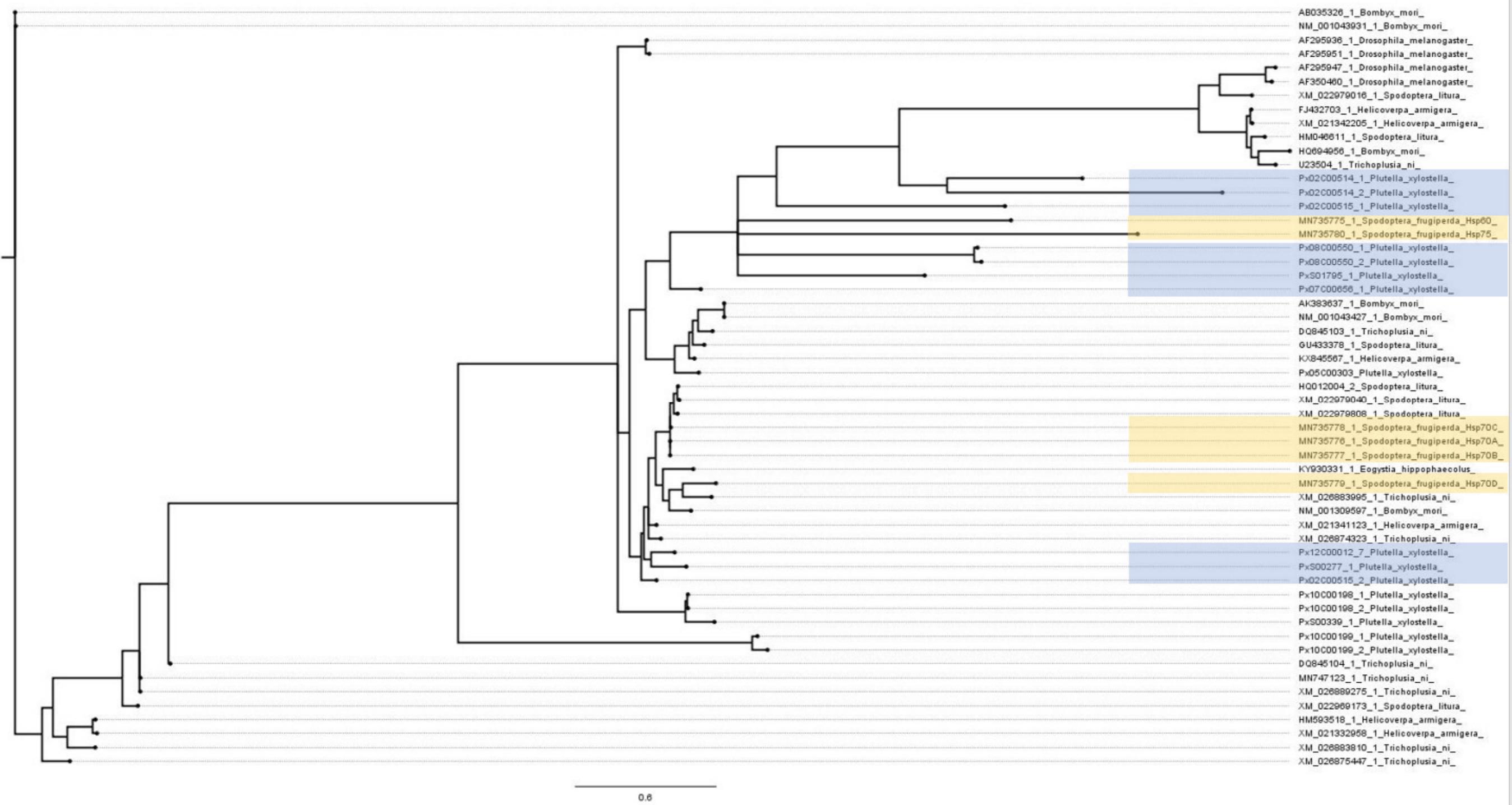
Primer	Sequence (5'→3')
Oligo-shEGFP-1	TAGTGTACAACATAAACAGCCACAAGTGTGCTG
Oligo-shEGFP-2	AGGACAGCACACTTGTGGCTGTTAGTTGTAC
Oligo-shEGFP-3	TCCTTGTGGCTGTTAGTTGTACTTTT
Oligo-shEGFP-4	AAACAAAAAGTACAACATAAACAGCCACA
EGFP(qPCR)-F	GAACCGCATCGAGCTGAAGG
EGFP(qPCR)-R	CTGCCGTCTCGATGTTGTG
RPL32(qPCR)-F	CAATCAGGCCATTACCGC
RPL32(qPCR)-R	CTGGGTTACGCCAGTTACG

Table S8. Primers for construction of pU6-gRNA plasmid and amplification assays.

Primer	Sequence (5'→3')
Oligo-sgRNA(EGFP)-1	TAGTGGCGAGGGCGATGCCACCTA
Oligo-sgRNA(EGFP)-2	AAACTAGGTGGCATGCCCTGCC
Oligo-sgRNA1(Ubx)-1	TAGTGGTGGTGGCGAGCAGCAGAA
Oligo-sgRNA1(Ubx)-2	AAACTTCTGCTGCTGCCACCAAC
Oligo-sgRNA2(Ubx)-1	TAGTGGATTGCGCTTACGACCGT
Oligo-sgRNA2(Ubx)-2	AAACACGCGTCGTAAGGCGAATCC
pU6-detection-R	TCACCAGCGTTCTGGGTGAG
Ubx-detection-F	CGGGGACCAGTACCGGGGGTTC
Ubx-detection-R	AGAGGAAGAAATCATGGGCTG



**Figure S1.** A phylogenetic tree of polyubiquitin (*Pub*) genes from different species. The corresponding tree scale is provided in the figure. The region covered in orange is the *Pub* gene with a strong promoter identified in *Aedes aegypti*. The blue area is the selected *Plutella xylostella* endogenous gene with a strong promoter.



**Figure S2.** A phylogenetic tree of heat shock protein 70(Hsp70) genes from different species. The corresponding tree scale is provided in the figure. The region covered in orange is the *HSP70* gene with a strong promoter identified in *Spodoptera frugiperda*. The blue area is the selected *P. xylostella* endogenous gene with a strong promoter.

-1267 CGGTACCTATCTCAAGGCACAGTCTAGTTAAATAATCAGCTAGACACGACAGATAGATCTTCGCCCCATTGTATAA  
Sp1  
-1187 GCCATTATTATTAGGAAGG\_AAGATATAAATTAAAGAAAATGTACAATGTTGGTTCGTCGCAACAGCGATGTTATT  
C/EPalp TBP  
-1107 TTCTGAGATCAGAATTACCAACATTACTGGCTGTACTATGTACTCTGTGATCATGAGTTGCACCTTGCTCTCAAG  
v-Myb C/EPalp  
-1027 ACATGACGCATGCGTTACTAGAAAAATCAAATAATCTGCTGTCTTCTGTTGTGCTAAAGTGTAAAAGGGAGACGGAT  
C/EPalp TBP  
-947 AAACAACGGCACCAATCAAACACAGTTATTGATTAAGTGTCAAGTCATATAAAAGCGAGGCAACTATTTCTGACGCAT  
C/EPbeta TBP  
-867 **TTC**AAAGTGAAAAATTAAACGCATCGCATAGTAAACTCACTGTTGTTGAAGATTTTATTGTACAAGAACAGACATT  
HNF-3  
-787 TCCTTGATAAATTATAGTGAATATTGGTAAGT TTCTTCCATTCTAACTACTCATATAACACCATACTTAAATTAGGC  
C/EPalpha  
-707 TGAATCTCAAACCAACTACATTTCGCTGGCTGAGAACTGCGCAGTGCAGCGCATTCAATTGATTCTTTCTT  
E1  
-627 TGTCGCCATCTAATCATCTGCTGGAAAATAATTGAATCACACCATCGTTATGCCCTCATCAAGGATTGAATAAC  
C/EPalp Sp1  
-547 ACAATTATTGTGGAAATGTTATTGATGATAAACCAACTTAGATTCTCGGATTTTATATAAATTGCAAAACTCTACAATA  
TBP  
-467 GGTGTGGCAAGGTTAATATAACAGCAATTACTGCAGAATTTCCTAAATATTGTAGCTTGCCTAACCTCGTTGGTT  
GCBox NF-1  
-387 GTGGATCATTTGGGTTATTCATATATAGGTATTACTACCTACGTAGTTACAGCAGTACCTACCTATCAAGTA  
GATA-1  
-307 ACCCAGAAGAAATAGGTATAAATAGGTACCTATAAACAATATTATGTTCAGTGTACGTTAGGTATTCTAAAGTAG  
CDC5 Evi-1 TBP  
-227 GTTTTAGGTCATTGATTGAATAGTTCCCTGACCTGTGCAAAACTCCGTTGGCTCAGCGCCATTATTATGACGCAGA  
GAATbox  
-147 CATGTTATGATGACAATTCAAAATATTGTGAAATCTTATCTTAAACACTTATTGAAATAAGTAGGTATGTTACG  
Mat1-Mc TBP  
-67 AATAGTGTTATCATCATTGGTGATAAAGGGATATTAACATTTTATTCCATTGTTACAGATCAAGATGCAGATCTTG  
TATABox +1  
+14 TCAAGACATTGACTGGCAAGACCATCACTCTAGAGGTGGAGCCTGCTGACACCATCGAGAATGTGAAGGCTAAGATTCAG

**Figure S3.** Characterization of the 217-2 promoter. Potential transcriptional elements in the sequences are predicted by using Genomatix Software Suit and Alibaba 2.0 databases. The bolded sequence part is the transcriptional regulatory region of the core of the 217 promoter, and the ATG marked in red is the translation initiation site of the *Px000217* gene.