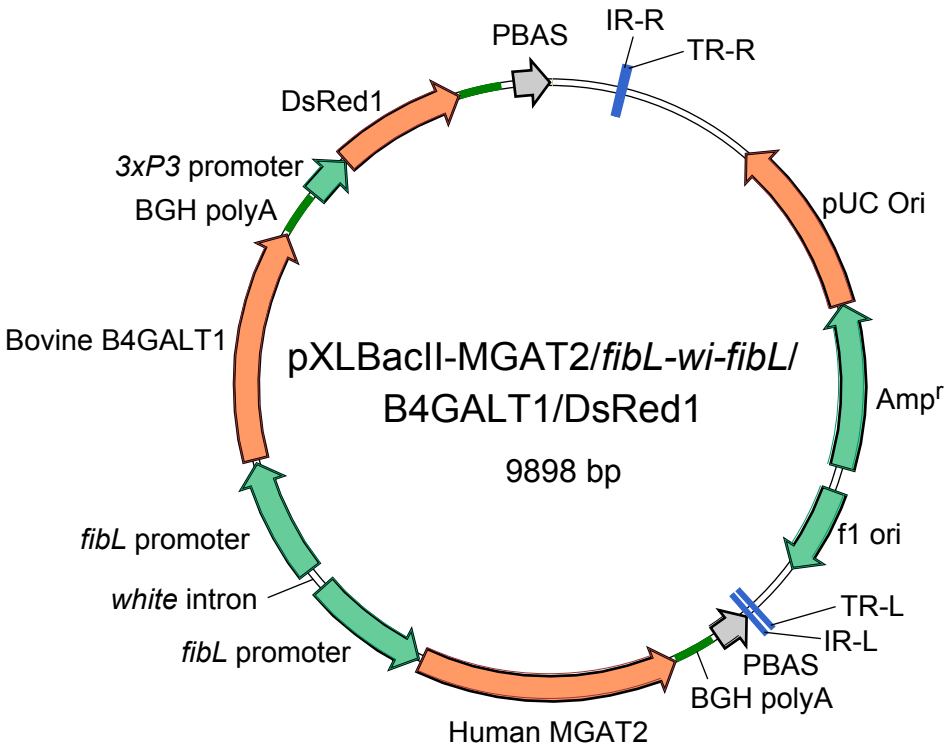

Fig. S1. *piggyBac* vector. This new dual *piggyBac* vector encodes both human MGAT2 and bovine B4GALT1 under the transcriptional control of the *Bombyx mori fibL* promoter, with each expression cassette separated by the *Drosophila white* intron 2 sequence. The vector also includes DsRed1 under the control of the neural-specific synthetic promoter, 3xP3, as an eye color marker. PBAS: *piggyBac* activation sequence, IR: inverted repeat, TR: terminal repeat.

Supplementary Material and Methods

1. Splinkerette PCR
    1. Prepare Splinkerette adaptors
        (1) Two different Splinkerette adaptors were prepared. One adaptor has GATC overhang, and the other adaptor has CTAG overhang. For GATC overhang adaptor, 7.5 μg of Splink-GATC-top primer and 7.5 μg of Splink-bottom primer were mixed with Buffer2 (New England Biolabs). For CTAG overhang, 7.5 μg of Splink-CTAG-top primer and 7.5 μg of Splink-bottom primer were mixed with Buffer2 (New England Biolabs). Both DNA mixtures were heated at 90°C for 5 min and cooled down for 30 min at room temperature.
    2. Prepare Splinkerette PCR template
        (1) Posterior silk glands (PSGs) from 4th day of 5th instar of FlcGG silkworms were dissected out. Genomic DNA of PSGs was prepared using Wizard genomic DNA purification kit (Promega).
        (2) 1 μg of genomic DNA was digested with *Bam*HI, *Bgl*II, *Bst*YI, *Spe*I, or *Xba*I for 3 hours at 37°C except for *Bst*YI (*Bst*YI was used at 65°C for 3 hours). After digestion reactions, digested genomic DNA fragments were purified with DNA mini-spin columns (Omega Biotech).
        (3) The genomic DNA digested with *Bam*HI, *Bgl*II, or *Bst*YI was ligated to Splinkerette adaptor with GATC overhang, and the genomic DNA digested with SpeI or XbaI was ligated to Splinkerette adaptor with CTAG overhang using T4 DNA ligase (NEB). These DNA ligates were used for the template for 1st Splinkerette PCR.
    3. 1st Splinkerette PCR
        (1) 1st Splinkerette PCR template was mixed with Splink1 primer and PB_L-1 primer for left side of *piggyBac*, or PB_R-1 for right side of *piggyBac*, and Phusion *Taq* polymerase (New England Biolabs) was used for PCR.
        (2) PCR was performed with 2 cycles of 98°C for 20 sec and 64°C for 15 sec, and 30 cycles of 98°C for 20 sec, 66°C for 15 sec, and 72°C for 2 min after the initial 1 min heating at 98°C.
        (3) The 1st Splinkerette PCR products were diluted at 1/50 with distilled water and used for the templates for 2nd Splinkerette PCR.
    4. 2nd Splinkerette PCR
        (1) 2nd Splinkerette PCR template was mixed with Splink2 primer and PB_L-2 primer for left side of *piggyBac*, or PB_R-2 for right side of *piggyBac*.
        (2) PCR was performed with 30 cycles of 98°C for 20 sec, 66°C for 15 sec, 72°C for 2 min after 1 min of 98°C incubation.
    5. Determine the sequences of Splinkerette PCR products
        (1) The 2nd Splinkerette PCR products were separated in 1% of agarose gel and purified with Agarose gel extraction kit (Omega Biotech).
        (2) dATP was added to the both ends of PCR products with the mixture of dNTP and *Taq* polymerase (New England Biolabs).
        (3) The PCR products were ligated to pGEM-T vector (Promega).
        (4) DH5α competent *E. coli* were transformed with the DNA ligates.
        (5) The positive clones for each PCR product were isolated by *Nde*I and *Sac*II digestion.
        (6) The insert DNAs were sequenced using T7 promoter primer and SP6 primer.
    6. Map the localization of *piggyBac* insertion sites in silkworm genome

(1) According to the sequencing results, the *piggyBac* insertion sites were determined by BLAST search at SilkDB: http://silkworm.genomics.org.cn/, and KAIKObase: http://sgp.dna.affrc.go.jp/KAIKObase/.

7. Sequences of primers used for Splinkerette PCR

Primer name	Sequence from 5' to 3'
Splink-GATC-top	gatcccactagtgtcgacaccagtctctaatttttttttttcaaaaaaa
Splink-CTAG-top	ctagccactagtgtcgacaccagtctctaatttttttttttcaaaaaaa
Splink-bottom	cgaagagtaaccgttgctaggagagaccgtggctgaatgagactggtgtcgacactagtgg
Splink1	cgaagagtaaccgttgctaggagagacc
PB_L-1	cgtcaattttacgcatgattatctttaacg
PB_R-1	cgtacgtcacaatatgattatctttctaggg
Splink2	gtggctgaatgagactggtgtcgac
PB_L-2	gactgagatgtcctaaatgcacagcg
PB_R-2	gagcaatatttcaagaatgcatgcgtc

Supplementary Table S1. Sequences of primers used in this study.

Primer name	Sequence (5' to 3')
hMGAT2-Fw	GGTGCATCAATGCTGAGT
hMGAT2-Rv	TGCATACCACAGTCTCCA
bB4GALT1-Fw	TGAGTTTAACATACCTGTGGAC
bB4GALT1-Rv	CCCCAGTAGTTATTAGGAAATC
Flc promoter-Fw	ATCGATGCCAAGTTACGTCAGTCGTATT
Flc promoter-Rv	TCGCGATTTAGTGGTCTGTTATGTGACC
BmRPL3-Fw	CGTCGTCATCGTGGTAAGGTCAAG
BmRPL3-Rv	GGTCTCAATGTATCCAACAACACCGAC
oligo$(dT)_{31}$-VN	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN