

Supplementary Information

Figure S1. Western blot analysis of GUS protein level in transgenic tobacco leaves. The antibody (ab50148) against GUS protein was purchased from Abcam Company (Shanghai, China). The second leaf from the apex of 40-day-old T₃ transgenic tobacco was collected as one sample from three independent transgenic lines. The samples were ground to powders in liquid nitrogen. The crude ptotein were extracted using sample buffer (S3401, Sigma, St. Louis, MO, USA). The supernatants were used for gel electrophoresis and Western blot. Lane 1–2: Crude protein from pWip₁₇₃₇ transgenic tobacco; Lane 3: Crude protein from pWip₁₅₀₀ transgenic tobacco; Lane 4–8: Crude protein from pWip₁₂₃₁ transgenic tobacco; Lane 9–13: Crude protein from pCAMBIA1300-221 transgenic tobacco; Lane 14: Crude protein from wide type tobacco; The target band was indicated by an arrow line.

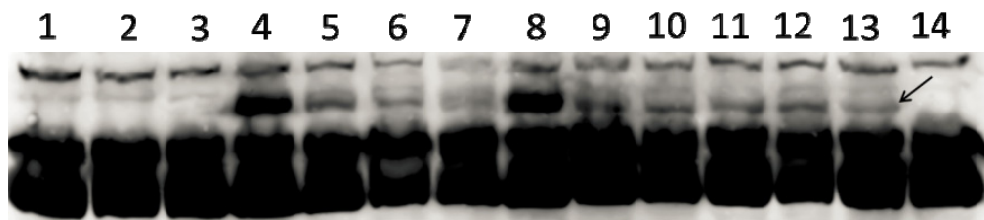


Figure S2. Analysis of enhancing translation motifs in 5'-UTR of *GUS* mRNA in pWip₁₂₃₁ transgenic tobacco. Part of *GUS* coding sequence was indicated in bold italic letters, and part of 5'-UTR from pCAMBIA1300-221 vector born was indicated in italic letters. CAA trimers were indicated in bold letters, and the ACAAAA motif and motifs similar to ACAATTAC element were shown with underline.

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CCTGATTAGATCGAGAGGGGAAGATTATTACTATACACTGTACATCACACTATATTCCATTGTT
TTACCTCATTGTGAATCGCACGCATGAAATGGTGAATAAGGCTAGGATCAATCAACCGATGCT
TCTACCAGCTGCGCGTCAAGCCCAAAACTGTGCTGATCGGATAACACGTCGTCGTCACCTC
CGTCCATTTTGGACCAATACATACGCTGATGAGGATGATCGGAAATGATCACGAGGAAGGA
TCCCCGGGTGGTCAGTCCCTTATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAA
ACTC

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Figure S3. uORF analysis of GUS mRNA in pWip₁₂₃₁ transgenic tobacco. Part of GUS coding sequence was indicated in bold italic letters, and part of 5'-UTR from pCAMBIA1300-221 vector bone was indicated in italic letters. Every uORF was underlined, and the one form by the uATG in frame with initiating ATG of *GUS* gene was underlined by green arrow line.

CCTGATTAGATCGAGAGGGGAAGATTATTACTATACACTGTACATCACACTATATTCCATTGTT

TTACCTCATTGTGAATCGCACGCATGAAATGGTGAATAAGGCTAGGATCAATCAACCGATGCT

TCTACCAGCTGCGCGTCAAGCCACAAAAGTGTGCTGATCGGATAACACGTCGTCGTCACCTC

CGTCCATTTTGGACCAAATACATACGCTGATGAGGATGATCGGAAATGATCACGAGGAAGGA

TCCCCGGGTGGTCAGTCCCTT**ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAA**

ACTC
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Figure S4. uORF analysis of *GUS* mRNA in pWip₁₇₃₇ transgenic tobacco. Part of GUS coding sequence was indicated in bold italic letters, and part of 5'-UTR from pCAMBIA1300-221 vector born was indicated in italic letters. Every uORF was underlined, and the uORF starting from the same uATG which is in frame with the initiating ATG of *GUS* gene in pWip₁₂₃₁ transgenic tobacco, was underlined by red line.

CCTGATTAGATCGAGAGGGGAAGATTATTACTATACACTGTACATCACACTATATTCCATTGTTTTACCTC

ATTGTGAATCGCACGCATGAAATGGTGAATAAGGCTAGGATCAATCAACCGATGCTTCTACCAGCTGCG

CGTCAAGCCACAAAAGTGTGCTGATCGGATAACACGTCGTCGTCACCTCCGTCATTTTGGACCAAATA

CATACGCTGATGAGGATGATCGGAAATGATCACGAGGAAGAGATGGTTCGTTATGCCATACGACTCGC

CGCCATGAGTCAAAGTTCCGCCTAACAAAATGTATGAGCTGGAAGCGTCTATCCATCCATCGCCCCCA

AAGAAATGCCATTGCAGGAGGACTACACGGCGGCAGGCAATGCCATGCATGTTAGATCTCGGCGCC

GCCTATAAGTAGCTTCTCCAGTGCACTGCTGTTGGCGCAACGAAGCTTGCTTCTACGAGAGGATATAG

AGAGGAAGAACAGCTCGTCCGAGACCGGATCCCCGGGTGGTCAGTCCCTT**ATGTTACGTCCTGTAGA**

AACCCCAACCCGTGAAATCAAAAACTC

Figure S5. uORF analysis of *GUS* mRNA in pWip_{1231a} transgenic tobacco. Part of *GUS* coding sequence was indicated in bold italic letters, and part of 5'-UTR from pCAMBIA1300-221 vector born was indicated in italic letters. Every uORF was underlined, the uORF starting from the same uATG which is in frame with the initiating ATG of *GUS* gene in pWip₁₂₃₁ transgenic tobacco, was underlined by red line. The uORF underlined by green arrow line could produce fusion protein with *GUS*.

CCTGATTAGATCGAGAGGGGAAGATTATTACTATACACTGTACATCACACTATATTCCATTGTTTACCTC

ATTGTGAATCGCACGCATGAAATGGTGAATAAGGCTAGGATCAATCAACCGATGCTTCTACCAGCTGCG

CGTCAAGCCACAAAACCTGTGCTGATCGGATAACACGTCGTCGCTCACTTCCGTCCATTTGGACCAAATA

CATACGCTGATGAGGATGATCGGAAATGATCACGAGGAAGAGATGGTTCGTTATGCCATACGACTCGC

CGCCATGAGTCAAAGTCCGCTAACAAAATGTATGAGCTGGAAGCGTCTATCCATCCATGGATCCCCG

GGTGGTCAGTCCCTTATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAACTC

Figure S6. The diagram of T-DNA region in expression vector pWip₁₇₃₇.

