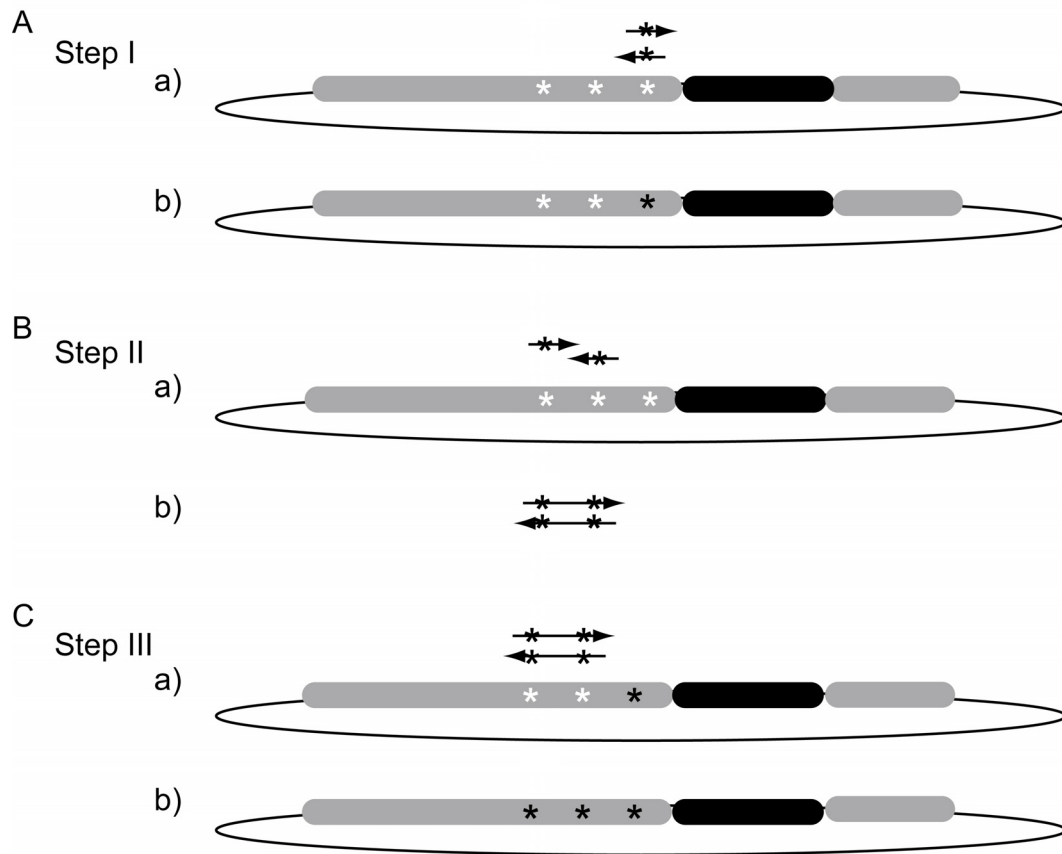


Supplemental Figure 4



Supplemental Figure 4. A Schematic Diagram of the Construction of *ProPUCI2.5m3:GUS*.

(A) Step I. The introduction of a point mutation into the AuxRE closest to the start codon in the 4.4-kb *PUCI* genomic fragment in pBluescript II (*gPUCI4.4*) using the QuikChangeTM method (Wang and Malcolm, 1999). The mutagenic primers (arrows) and

gPUCHI4.4 are indicated in (a), and the single mutant product in (b).

(B) Step II. A mutagenized 514-bp PCR fragment was amplified from *gPUCHI4.4* using a PCR to generate a mutagenic mega primer pair. The mutagenic primers (arrows) and *gPUCHI4.4* are indicated in (a), and the PCR product in (b).

(C) Step III. Generation of the triple-mutated promoter by using the single-mutated *gPUCH4.4* product from Step I as a template for the PCR and the mutated mega primer pair from Step II. The mutant product from Step I and the mutagenic mega primers from Step II (arrows) are indicated in (a), and the triple-mutated product in (b).

The white stars indicate the wild-type AuxREs, whereas the black stars denote the mutant AuxREs. Black bars indicate the *PUCHI* ORF and gray bars indicate the 5' and 3' flanking sequences of the *PUCHI* ORF.