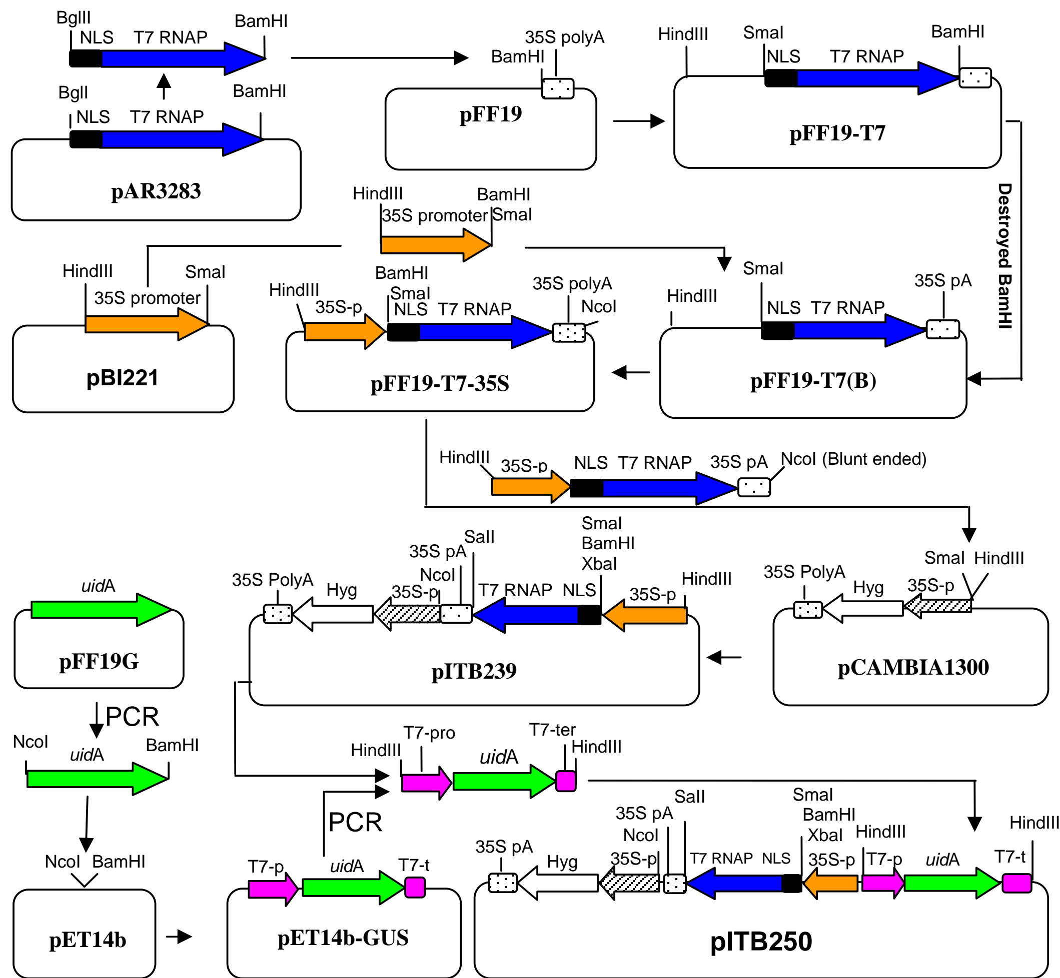

Figure 1. Construction of pITB250.

The 2.7 kb *BglII-BamHI* fragment of T7 RNA polymerase (T7 RNAP) with nuclear localization signal (NLS) from pAR3283 was cloned into *BamHI* site of pFF19 (pFF19 was created from pFF19G by removed GUS gene by digestion with *PstI* and religation) to yield pFF19-T7. The *BamHI* in pFF19-T7 was destroyed by digestion with *BamHI* followed by filling with the klenow enzyme and religation to yield pFF19-T7(B). The "35S enhancer-promoter" in pFF19-T7(B) was replaced with CaMV 35S promoter from pBI211 (Clontech) using *HindIII-SmaI* enzymes to yield pFF19-T7-35S(B). The *HindIII-NcoI* (*NcoI* blunt ended with klenow) fragment containing "35S promoter:T7RNAP:35S polyA" from pFF19-T7-35S(B) was cloned into *HindIII-SmaI* site of pCAMBIA1300 to yield pITB239. Note that after ligation *SmaI* site was lost but *NcoI* site is recreated. The *uid*A gene was amplified from pFF19G and cloned into *NcoI-BamHI* sites of pET14b (Novagen). The *BamHI* site was destroyed by digestion with *BamHI* followed by filling with klenow and religation to yield pET14b-GUS. The 2.1 kb *HindIII-HindIII* fragment containing "T7 promoter:*uid*A:T7 terminator" was amplified from pET14b-GUS and cloned into *HindIII* site of plasmid pITB239 to yield pITB250.