



**Figure 2.** PCR and restriction digestion analysis to confirm various cloning steps in the construction of pITB239 and pITB250 vectors (see figure 1 for other details).

- A.** *HindIII* digested  $\lambda$  DNA marker
- B.** Digestion pFF19 (lane 1) and pFF19-T7 (lane 2) with *SmaI-BamHI* determined that the T7 RNAP was inserted into pFF19. M: Marker  $\lambda$  *HindIII*.
- C.** Digestion of pFF19-T7 (lane 1) and pFF19-T7(B) (lane 2) with *BamHI-SmaI* determined that *BamHI* in pFF19-T7(B) was destroyed. The unique *BamHI* site was destroyed by restriction digestion, end filling and religation.
- D.** Digestion of pFF19-T7(B) (lane 2) and pFF19-T7-35S (lane 1) with *HindIII-BamHI* to determine replacement of CaMV 35S promoter with “35S promoter -enhancer”. In pFF19-T7(B) the *BamHI* site was destroyed, thus if it has exchanged, other *BamHI* site come from CaMV 35S promoter, a band of ~800 bp should drop out (lane 1).
- E.** Digestion of pET14b-GUS (lane 1) and pET14b (lane 2) with *NcoI-BamHI* showed the presence of 1.8 kb *uidA* gene cloned into pET14B.
- F.** Digestion of pCAMBIA1300 (lane 2) and pITB239 (lane 1) with *HindIII-Sall* showed that the “35S promoter:T7RNA:35S polyA” cassette was inserted into pCAMBIA1300.
- G.** Digestion of pITB239 (lane 4) and pITB250 (lane 3) with *HindIII* to determine insertion of “T7-promoter:*uidA*:T7 terminator” cassette into pITB239. Digestion of pITB239 (lane 1) with *HindIII-Sall* and pITB250 (lane 2) with *BglII-Sall* to determine the insertion of *uidA* in clockwise direction.