



**Figure 12.** PCR and restriction digestion analysis to confirm various cloning steps in the construction of pITB441 and pITB450 vectors (see figure 11 for other details).

**A.** *HindIII* digested  $\lambda$  DNA marker

**B.** Digestion of pFF19 (lane 1) and pFF19-Glu with *HindIII-KpnI* to determine the cloning of *GluB1* promoter into pFF19 by replacing “35S promoter-enhancer”. M: Marker

**C.** Digestion of pFF19-Glu (lane 1) and pFF19-Glu-T7 (lane 2) with *KpnI* showed the insertion of T7 RNAP into pFF19-Glu.

**D.** Digestion pFF19-Glu-T7 (lane 1) and pFF19-Glu-T7(B) with *BamHI-HindIII* shown *BamHI* site in pFF19-Glu-T7(B) was destroyed. The pFF19-Glu-T7 (B) was linear (a band ~7.6 kb), while pFF19-Glu-T7 has two bands (~3.6 kb and ~4.0 kb).

**E.** Digestion of pA4-Glu-T7(B) (lane 1) and pCAMBIA1300 (lane 2) with *HindIII-NcoI* to confirm the insertion of ~4.2 kb DNA “*GluB1* promoter:T7 RNAP” fragment into pCAMBIA1300.

**F.** Digestion of pET14b (lane 1) and pET14b-Fe (lane 2) with *NcoI-BamHI* to determine cloning of ~0.7 kb ferritin gen into pET14b.

**G.** Digestion of pA4-Glu-T7(B) (lanes 1) and pITB241 (lane 2) with *HindIII* to show the cloning of “T7 promoter :ferritin:T7 terminator” into pA4-Glu-T7(B). Digestion of pA4-Glu-T7(B) with *Sall-HindIII* (lane 3) and pITB241 with *Sall-BglII* (lane 4) showed the presence of a ~4.0 kb band that determined the insertion of the cassette in clockwise direction.

**H.** Digestion of pITB241 (lane 1) and pITB342 (lane 2) with *HindIII* showed the insertion of “T7 promoter:*uidA*:T7 terminator” cassette into pITB241. Digestion of pITB241 (lane 3) and pITB342 (lane 4) determined that the cassette was cloned in clockwise direction.