



**Figure 16.** Restriction analysis to confirm various cloning steps in the construction of pITB247 series.

- A.** . *HindIII* digested  $\lambda$  DNA marker
- B.** Digestion of pITB245 (lane 1) and pITB247 (lane 2) with *HindIII-BamHI* show that the “T7 promoter;HBsAg:T7 terminator” cassette was inserted into pITB245.
- C.** Digestion of pITB245 (lane 1) and pITB247 (lane 2) with *HindIII-BglII* that determined the orientation of HBsAb.
- D.** Digestion of pITB247 (lane 1), pITB247G3 (lane 2), pITB247G1(lane 3) and pITB247G2 (lane 4) with *HindIII-BamHI* to confirm the insertion of “T7 promoter:*uidA*:T7 terminator” cassette into pITB247. The results show that in pITB247G3 (lane 2) has two copies, pITB247G1( lane 3) and pITB247G2 (lane 4) have single copy inserted.
- E.** Digestion of above plasmids with *HindIII-BglII* to confirmed copy number of inserts and orientation. Colony in n lane 2 two copies of cassette (both in clockwise direction). Colonies in lane 3 and 4 have single copy in clockwise and anticlockwise orientation, respectively.