



Figure 14. Restriction digestion to confirm various cloning steps in the construction of pITB245 series (see figure 13 for other details).

- A.** *HindIII* digested λ DNA marker.
- B.** Restriction analysis to confirm the cloning of PDS promoter into pGEMT_Easy vector. Digestion of pGEMT-Easy-PDS with *BglIII* (lane 1) showed a linear of plasmid (~5.0 kb), while digestion with *BglIII-SmaI* (lane 2) showed the presence of 2.1 kb band corresponding to the size of PDS promoter. M: Marker.
- C.** Digestion of pITB245 with *SmaI-BglIII* (lane 1), *HindIII-BglIII* (lane 2), *HindIII-BamHI* (lane 3) and *Sall-BglIII* (lane 4) to determine the cloning of GluB1 promoter into pITB241 by replacing PDS promoter.
- D.** Digestion of pITB245 (lane 1), pITB245G1 (lane 2), pITB245G2 (lane 3) and pITB245G3 (lane 4) with *HindIII-BamHI* to show the cloning of “T7 promoter:*uidA*:T7 terminator” cassette into pITB245.
- E.** Digestion pITB245 (lane 1), pITB245G1 (lane 2), pITB245G2 (lane 3) and pITB245G3 (lane 4) with *HindIII-BglIII* that determined the orientation and copy number of cassette. Lanes 2 and 3 shown that one copy of cassette was inserted (in different orientations. Lane 4 shows the presence of two copies, both in anticlockwise direction.