



**Figure 18.** Restriction analysis to confirm the construction of pITB345 series

- A.** *HindIII* digested  $\lambda$  DNA marker
- B.** Digestion of pB4-T7-PDS (lane 1) and pCAMBIA1301 (lane 2) with *BamHI-NcoI* to determine the cloning of “PDS promoter:T7 RNAP:35S polyA” into pCAMBIA1301.
- C.** Digestion of pA8-T7-PDS(r) (lane 1) and pA8-T7-PDS(l) (lane 2) with *NcoI* to determine the orientation of “PDS promoter:T7 RNAP:35S polyA” in pA8-T7-PDS(r). The result showed that the cassette was inserted in anticlockwise direction.
- D.** Digestion of pA8-T7-PDS(l) (lane 1), pITB345 (lane 2) and pITB345D (lane 3) with *Sall* to determine the copy number. The result showed that the insertion of single copy in pITB345 and two copies in pITB345D.
- E.** Digestion of pA8-T7-PDS(l) (lane 1), pITB345 (lane 2) and pITB345D (lane 3) with *Sall-BamHI* showed the single copy insertion in pITB345 in anticlockwise direction and two copies in pITB345D (same orientation in anticlockwise direction); M: Marker.