



Figure 10. Restriction digestion to confirm various cloning steps in the construction of pITB741, pITB750, pITB841 and pITB850 vectors (see figure 9 for other details).

- A. Digestion of pGEMT-Easy-*pal1* (lane 1) and pGEMT-Easy-*pal1*Δ (lane 2) with *BglII-SmaI* that determined the cloning of *pal1* and *pal1*Δ into pGEMT-Easy.
- B. PCR to check replacement of *pal1* and *pal1*Δ promoter with *kin1* promoter in pITB650 and pITB641 to create pITB750 (lane 1), pITB741 (lane 2), pITB850 (lane 4) and pITB841 (lane 5). NC: Negative control, PC1: Positive control for *pal1*, PC2: Positive control for *pal1*Δ.
- C. *HindIII* digested λ DNA marker
- D. Digestion of pITB850 (lane1), pITB750 (lane 2), pITB650 (lane 3), pITB841 (lane 4), pITB741 (lane 5) and pITB641 (lane 6) with *HindIII-SmaI* to confirm replacement of *pal1* and *pal1*Δ promoter with *cor6.6* promoter in pITB650 and pITB641. Note a ~2.7 kb band in lane 1 corresponding to the size of “*pal1* promoter:*T7* promoter:*uidA*:*T7* terminator”, in lane 2, a ~3.0 kb band corresponding to the size of “*pal1*Δ promoter:*T7* promoter:*uidA*:*T7* terminator” and in lane 3 (pITB650), a 3.4 kb band corresponding to the size of “*cor6.6* promoter:*T7*-promoter:*uidA*:*T7* terminator”. Also note the presence of 0.6 kb in pITB841 (*pal1* promoter), 0.9 kb in pITB741 (*pal1*Δ promoter) and 1.3 kb in pITB641 (*cor6.6* promoter).