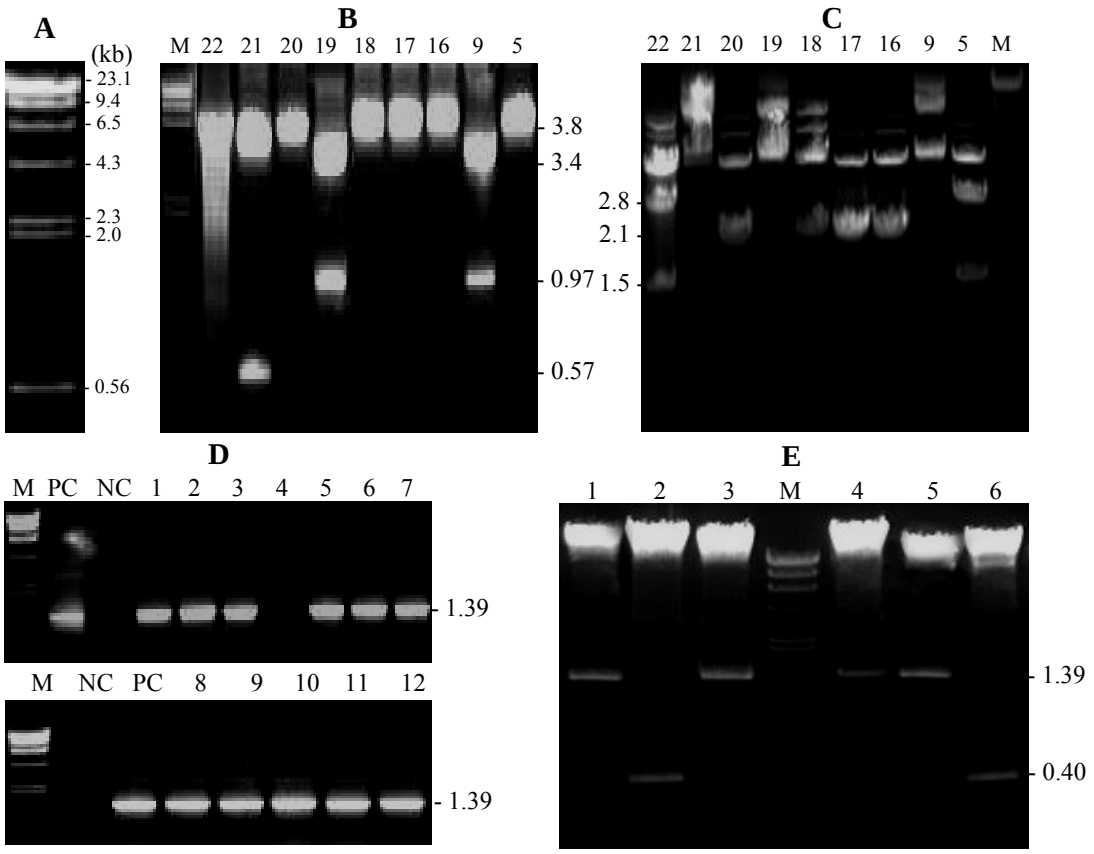

Figure 8. PCR and restriction digestion analysis to confirm various cloning steps in the construction of pITB541, pITB550, pITB641 and pITB650 vectors

A. *HindIII* digested λ DNA marker

B. PCR amplified *kin1* and *cor6.6* promoters containing DNA fragments were cloned into pGEMT-Easy to yield pGEMT-Easy-Kin1 and pGEMT-Easy-cor6.6, respectively (see figure 7). Insertion of *kin1* promoter was confirmed by digestion with *NsiI.* Colony # 9, 19 and 21 showed the insertion of *kin1* promoter. The pGEMT-Easy-kin1 has two *NsiI* sites: one in pGEMT-Easy (127) and the other one in *kin1* promoter (443). Colonies # 9 and 19 have insets in clockwise direction (two bands ~0.97 kb and ~3.4 kb) and colony 21 has insert in anticlockwise direction (~0.57 kb and ~ 3.8 kb bands); M: Marker .

C. Insertion of cor.6.6 promoter into pGEMT-Easy. Digestion with *SacI*. Colony # 5, 16, 17, 18 and 20 have showed the cloning of *cor6.6* promoter in both the orientations. The pGEMT-Easy after cloning cor6.6 will have two *ScaI* sites, one in pGEMT-Easy (1890), and the other in *cor6.6* promoter (352). The clockwise direction Colonies #5 and 22 have insert in clockwise direction and the colonies #16, 17, 18 and 20 have insert in anticlockwise direction.

D. PCR analysis confirmed the cloning of *kin1* and *cor6.6* promoter into pITB450 and pITB441 (Figure 3). Lanes 1, 2 and 3 show presence of *kin1* promoter in pITB550. Lanes 5, 6 and 7 show the presence of *kin1* promoter into pITB541. Lanes 8, 9 and 10 show presence of *cor6.6* promoter in pITB650. Lanes 11 and 12 show presence of *cor6.6* promoter in pITB641). M: *Hind III* λ marker; NC: Negative control; PC: Positive control.

E. Digestion of pITB550 (lane 1) , pITB450 (lane 2) and pITB650 (lane 3) with *SmaI-BglII* and pITB441 (lane 6), pITB541 (lane 4) and pITB641 (lane 5) with *BamHI-HindIII* to confirm insertion (see map in figure 7)