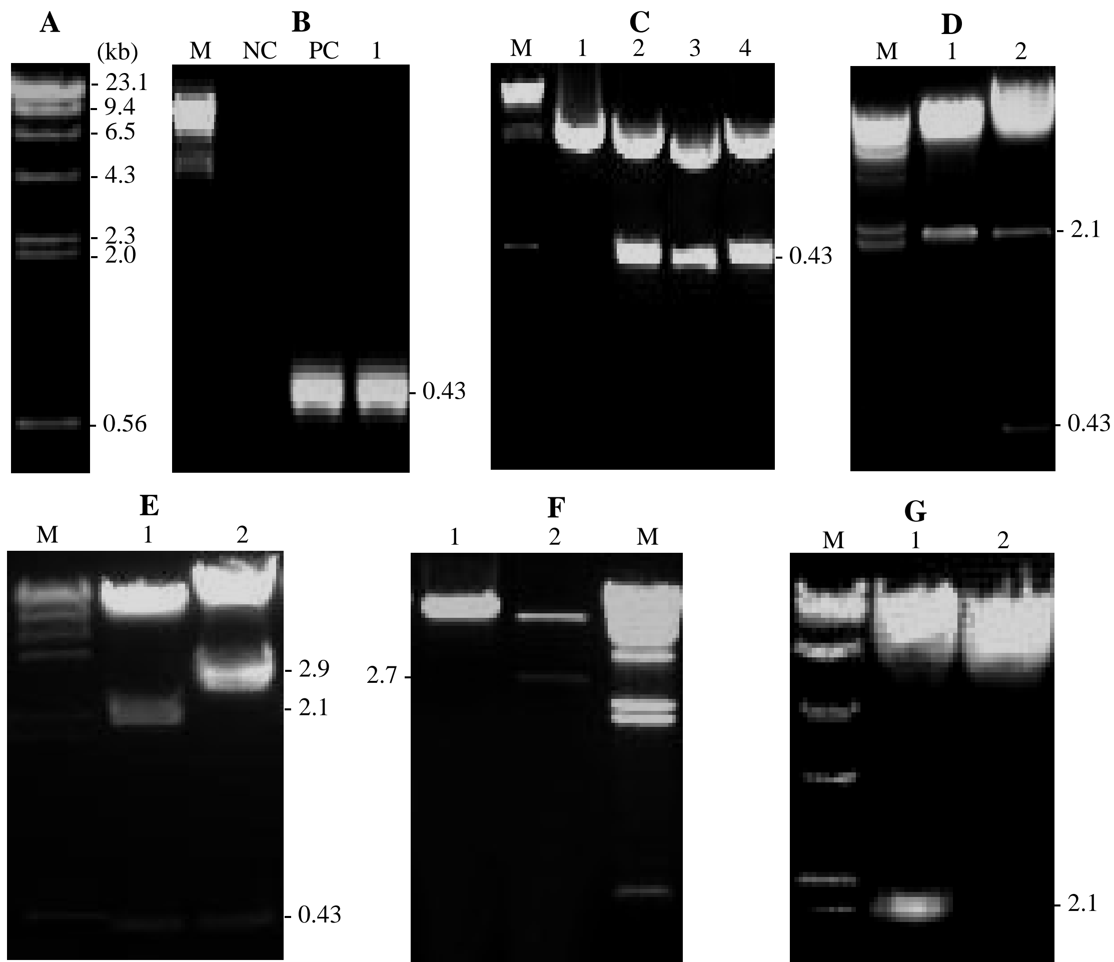

Figure 4. PCR and restriction digestion analysis to confirm various cloning steps in the construction of pITB441 and pITB450 vectors (see figure 3 for other details).

A. *HindIII* digested λ DNA marker

B. PCR amplification to check insertion of *rbc*S promoter into pGEMT-Easy (lane 1), NC: Negative control; PC: Positive control. M: HindIIIλ marker

C. Digestion of pGEMT-Easy-rbcS with *XbaI* (lane 2), *HindIII* (lane 3) and *BglII-SmaI* (lane 4) showed the presence of expected band corresponding to the size of *rbc*S promoter. Restriction digestion with *NcoI-BamHI* (lane 1) determined that insertion of *rbc*S promoter was clockwise in direction.

D. Digestion of pITB250 (lane 1) and pITB450 (lane 2) with *HindIII-BglII* that determined replacement of CaMV 35S promoter with *rbc*S promoter. Note the presence of a ~400 bp band in pITB450 representing the *rbc*S.

E. Digestion of pITB441 (lane 1) and pITB440 (lane 2) with *HindIII-NcoI* that determined the cloning of "*uid*A:35S polyA" by replacing "T7 RNAP:35S polyA" Note the presence of a ~ 2.1 kb band (*uid*A) in pITB441 and a ~2.9 kb band (T7 RNAP) in pITB440.

F. Digestion of pBin-HygTX (lane 1) and pBin-HygTX-T7 (lane 2) with *BamHI-SalI* that determined insertion of T7 RNAP into pBin-HygTX.

G. Digestion pITB228 (lane 1) and pBin-HygTX-T7 (lane 2) with *HindIII* determine that "T7promoter-*uid*A-T7 terminator" cassette was inserted into pBin-HygTX-T7.