

**Fig1.** PCR amplification of the 700 bp *oriT/mob* region in *C. difficile* transconjugants containing pMTL9301 lanes 2-7 (the DNA was amplified with primers ORT-1 and ORIT-2 table 2) and 300 bp region flanking the deleted *oriT/mob* region in *C. difficile* strains containing pMTL9301 $\Delta$ *oriT* lanes 9-12 (the DNA was amplified with *oriT* (F1050) and *oriT*(R2210). Template DNA in each lane is as follows: Lane 1) purified pMTL9301; 2) CA434::pMTL9301 x CD37 (+ DNase 50); 3) CA434::pMTL9301 x CD37 (No DNase); 4) CA434::pMTL9301 x 630 $\Delta$ *erm* (+ DNase 50); 5) CA434::pMTL9301 x 630 $\Delta$ *erm* (No DNase); 6) HB101::pMTL9301 x CD37 (No DNase); 7) HB101::pMTL9301 x 630 $\Delta$ *erm* (No DNase); 8) purified pMTL9301 $\Delta$ *oriT*; 9) CA434:: pMTL9301 $\Delta$ *oriT* x CD37 (No DNase); 10) CA434:: pMTL9301 $\Delta$ *oriT* x 630 $\Delta$ *erm* (+ DNase 50); 11) CA434:: pMTL9301 $\Delta$ *oriT* x 630 $\Delta$ *erm* (No DNase); 12) HB101:: pMTL9301 $\Delta$ *oriT* x 630 $\Delta$ *erm* (No DNase)