

FIG. 3.14 Coomassie blue staining and Western blot of nonstructural proteins expressed in *E. coli* NovaBlue(*DE3*). (A) Coomassie blue staining of the supernatants of NovaBlue(*DE3*) transformed with different plasmids. (B) Coomassie blue staining of the pellets of NovaBlue(*DE3*) transformed with different plasmids. (C) Western analysis with anti-HA antibody against supernatants of NovaBlue(*DE3*) transformed with different plasmids. (D) Western analysis with anti-HA antibody

against pellets of NovaBlue(*DE3*) transformed with different plasmids. (E) Western analysis with anti-His antibody against supernatants of NovaBlue(*DE3*) transformed with different plasmids. (F) Western analysis with anti-His antibody against pellets of NovaBlue(*DE3*) transformed with different plasmids. Lane 1, pET-30b-HeptB (positive control for His); lane 2, pKRY (positive control for HA); lane 3 and 8, pcDNA3 (negative control); lane 4 and 9, pNS2A-HAHis; lane 5 and 10, pNS2B-HAHis; lane 6 and 11, pNS4A-HAHis; lane 7 and 12, pcDNA3-4B-HAHis; M, marker.

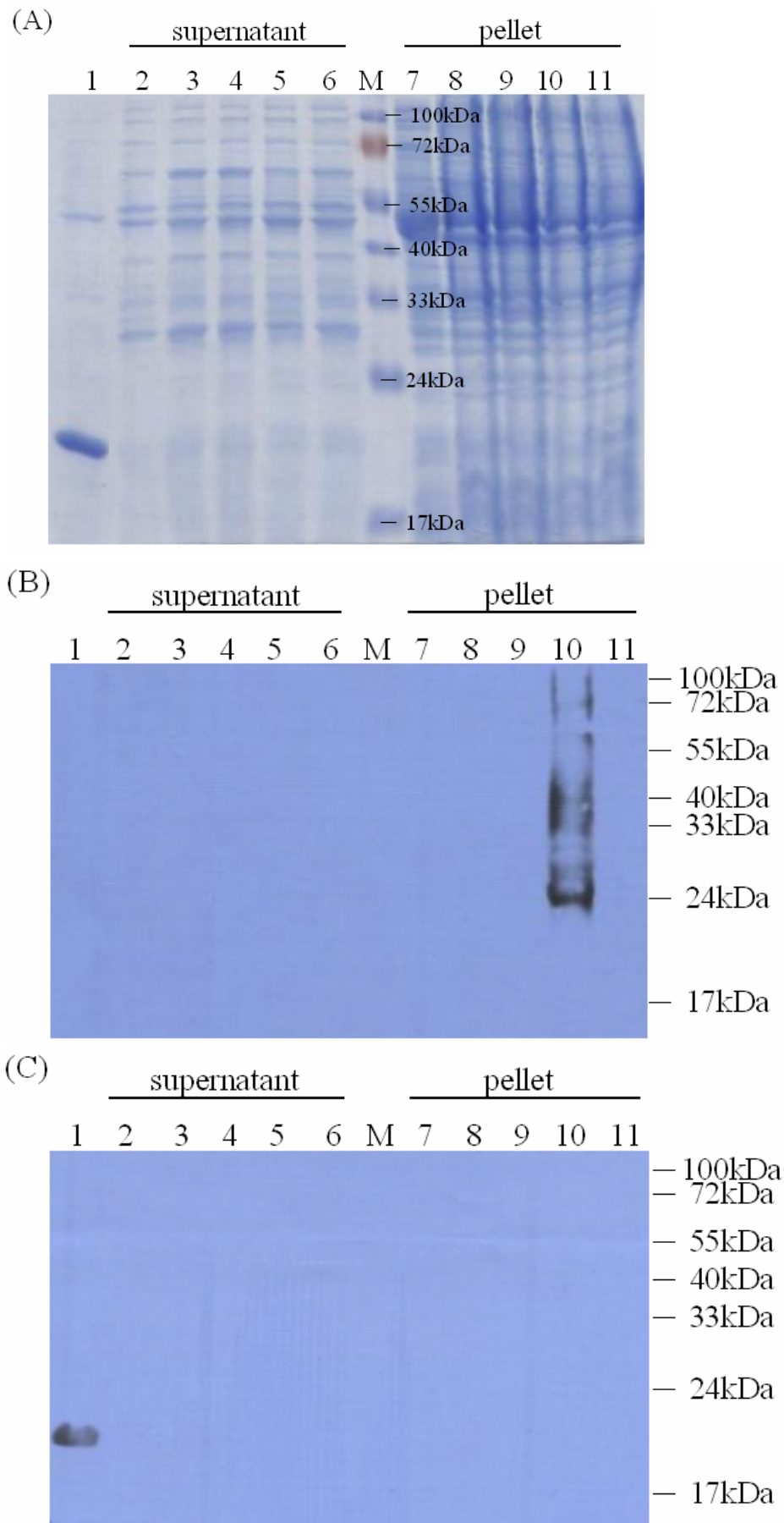


FIG. 3.15 *E. coli* NovaBlue(*DE3*) expressed nonstructural proteins analyzed by coomassie blue staining and Western blot. (A) Coomassie blue staining of the supernatant and pellet of NovaBlue(*DE3*) transformed with different plasmids. (B)

Western analysis with anti-HA antibody against supernatant and pellet of NovaBlue(*DE3*) transformed with different plasmids. (C) Western analysis with anti-His antibody against supernatant and pellet of NovaBlue(*DE3*) transformed with different plasmids. Lane 1, pET-30b-HeptB (positive control for His); lane 2 and 7, pcDNA3 (negative control); lane 3 and 8, pcDNA3(pro)-2A-HAHis; lane 4 and 9, pcDNA3(pro)-2B-HAHis; lane 5 and 10, pcDNA3(pro)-4A-HAHis; lane 6 and 11, pcDNA3(pro)-4B-HAHis; M, marker.

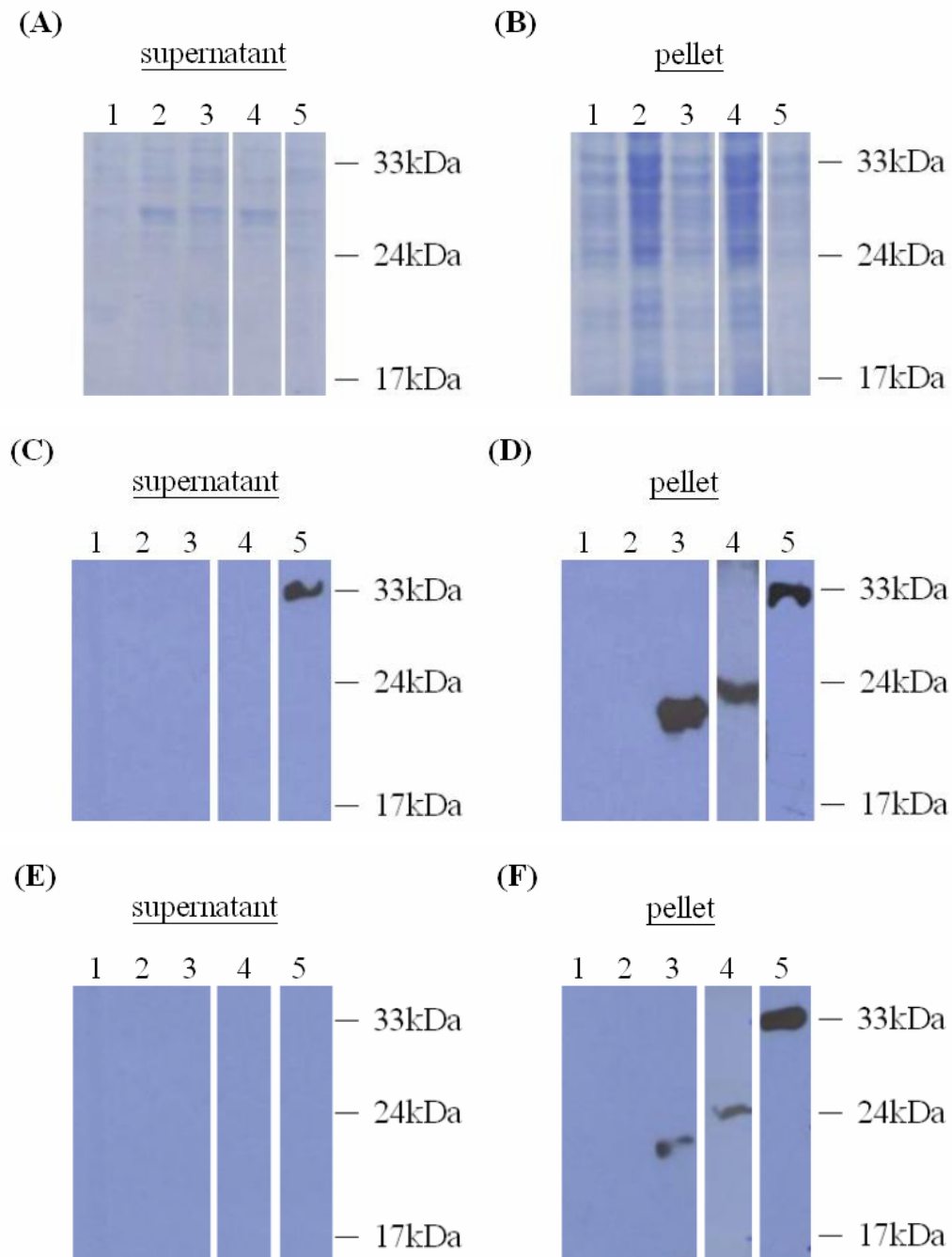


FIG. 3.16 Coomassie blue staining and Western blot analyzing nonstructural proteins expressed in *E. coli* NovaBlue(*DE3*). (A) Coomassie blue staining of the supernatants of NovaBlue(*DE3*) transformed with different plasmids. (B) Coomassie blue staining of the pellets of NovaBlue(*DE3*) transformed with different plasmids. (C) Western analysis with anti-HA antibody against supernatants of NovaBlue(*DE3*) transformed with different plasmids. (D) Western analysis with anti-HA antibody against pellets of NovaBlue(*DE3*) transformed with different plasmids. (E) Western analysis with anti-His antibody against supernatants of NovaBlue(*DE3*) transformed with different plasmids. (F) Western analysis with anti-His antibody against pellets of NovaBlue(*DE3*) transformed with different plasmids. Lane 1, pET-30a(+) (negative control); lane 2, pET Δ 5T-2A-HAHis; lane 3, pET Δ 5T-2B-HAHis; lane 4, pET Δ 5T-4A-HAHis; lane 5, pET Δ 5T-4B-HAHis.

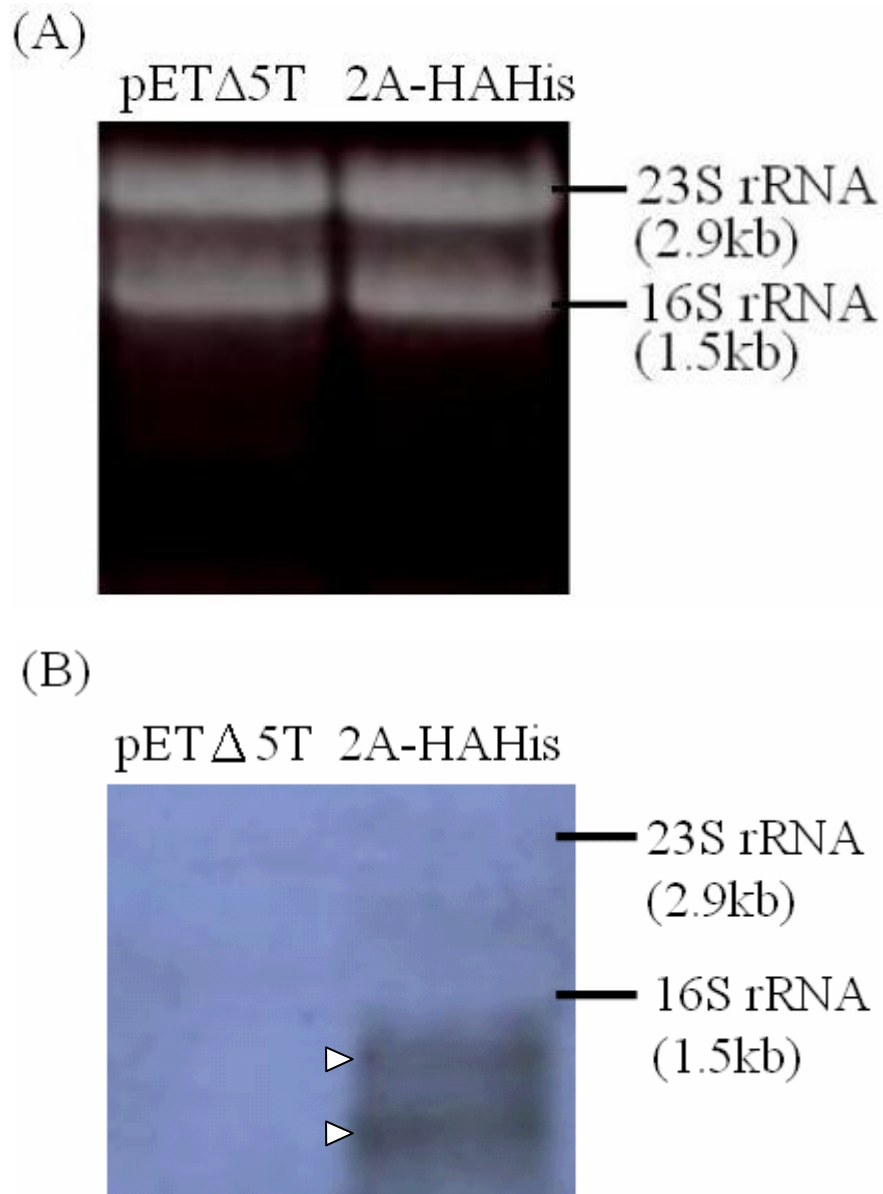


FIG. 3.17 Northern blot of NS2A mRNA expressed in *E. coli* NovaBlue(*DE3*). (A) RNA of NovaBlue(*DE3*) transformed with pET Δ 5T or pET Δ 5T-2A-HAHis before transfer. (B) Northern blot of mRNA expressed in NovaBlue(*DE3*) transformed with pET Δ 5T or pET Δ 5T-2A-HAHis.

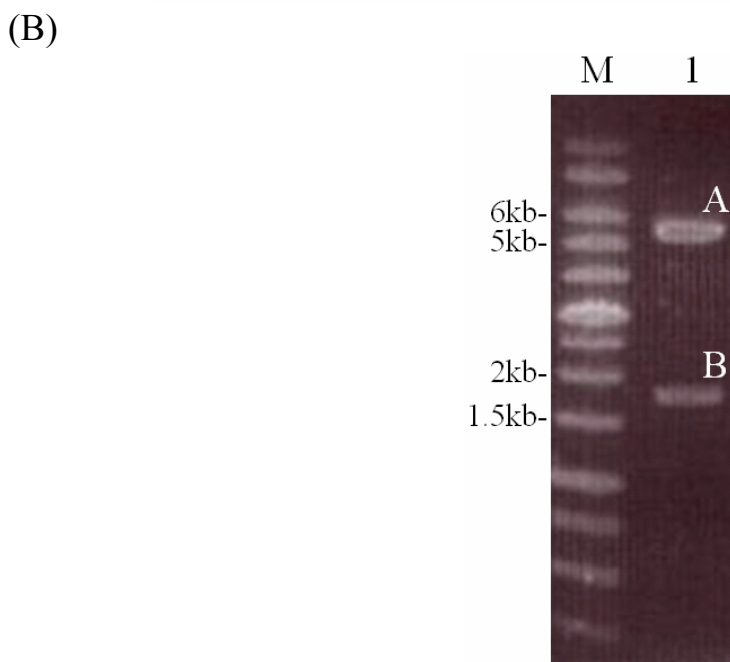
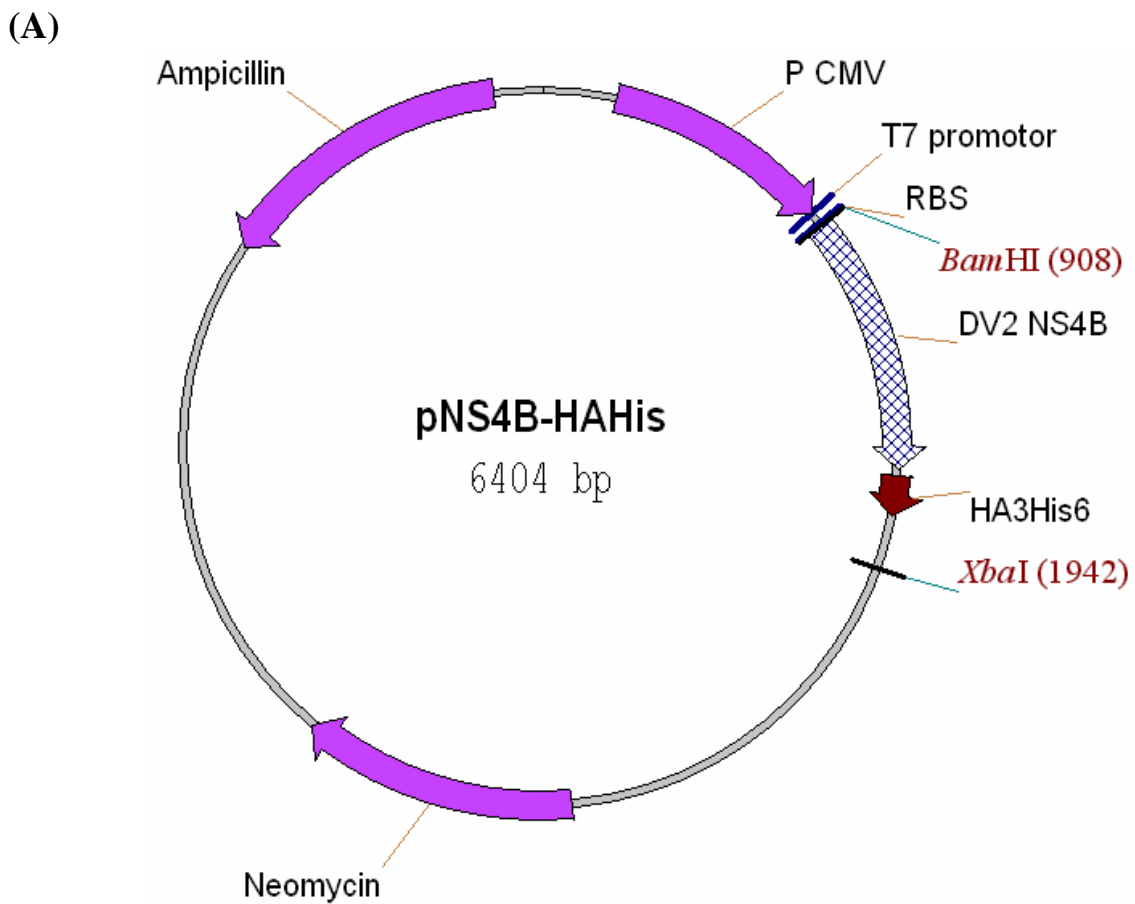
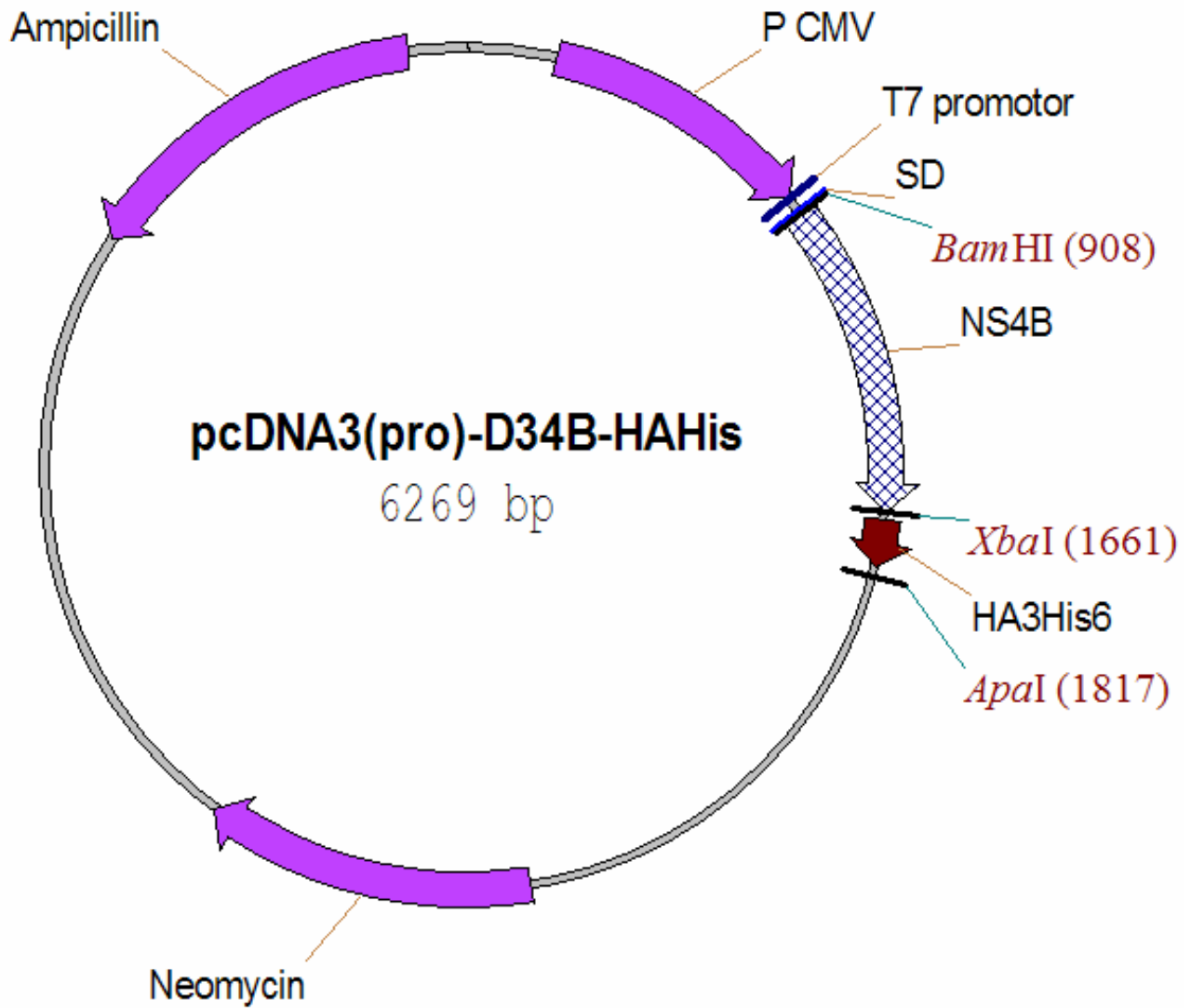


FIG. 4.1 Restriction enzyme digestion of pNS4B-HAHis. (A) Map of pNS4B-HAHis. (B) Lane 1, *Bam*HI and *Xba*I digested pNS4B-HAHis; M, marker. A, 5370bp; B, about 1.8kb (it should have been 1034bp theoretically).

Appendix 1

Map of pcDNA3(pro)-D34B-HAHis

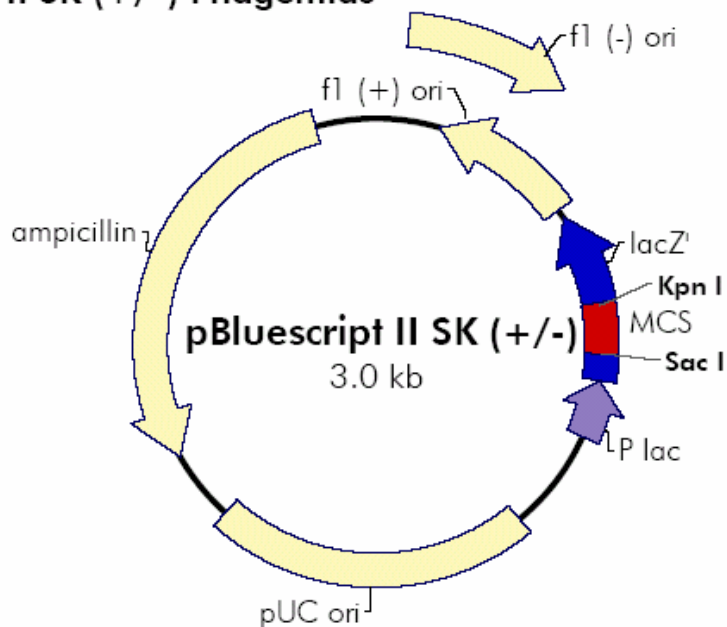


(From 陳欣悟, Yang laboratory)

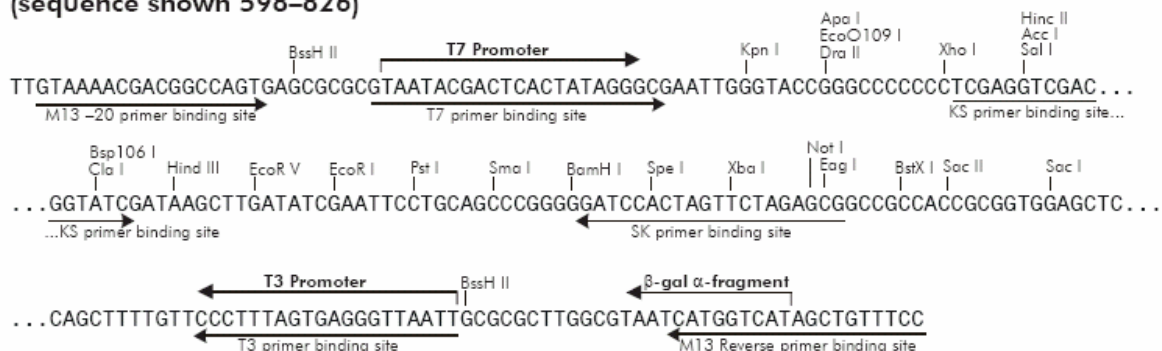
Appendix 3

Map of pBluescript II SK (+)

pBluescript® II SK (+/-) Phagemids



pBluescript II SK (+/-) Multiple Cloning Site Region (sequence shown 598–826)

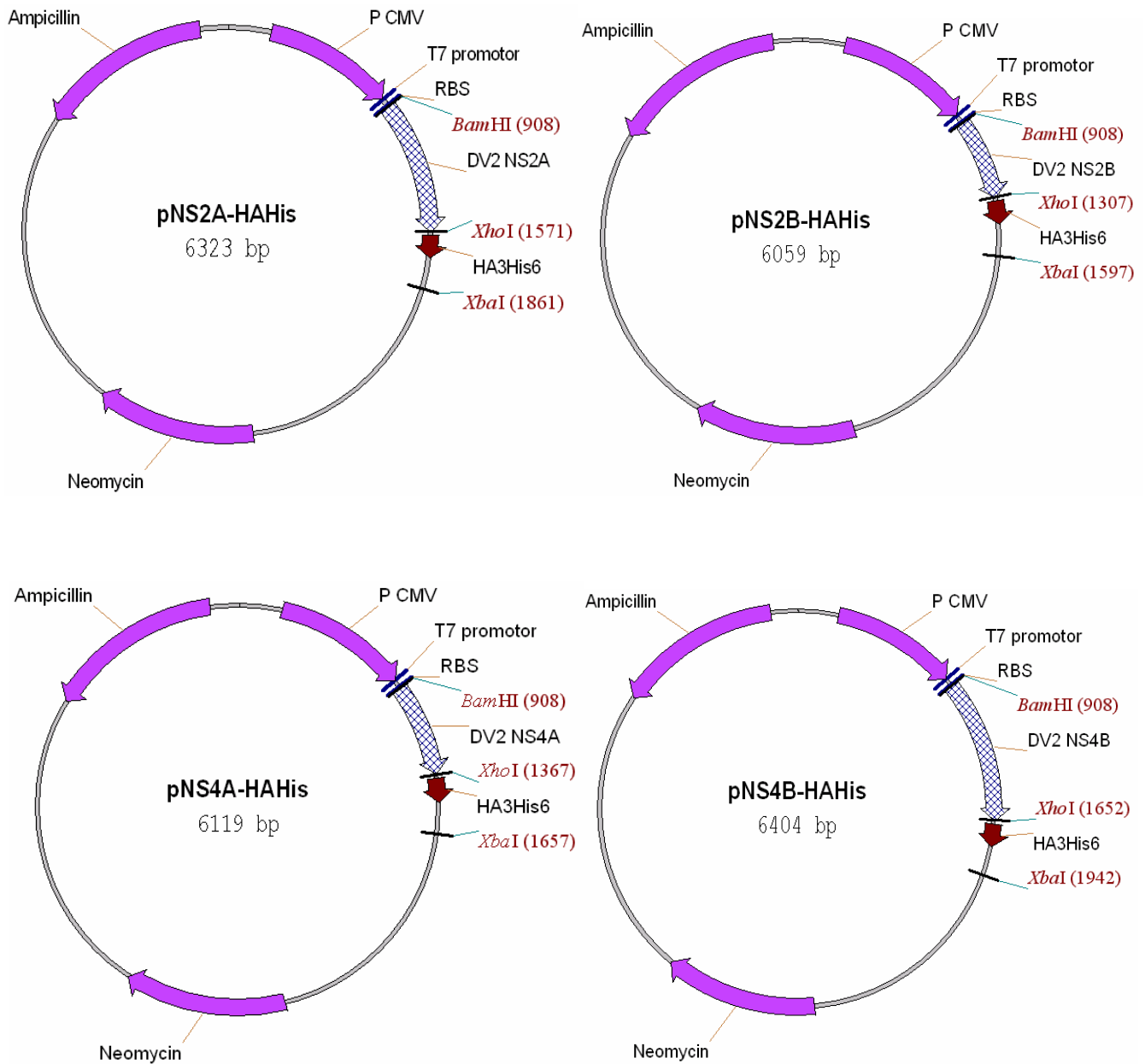


Feature	Nucleotide Position
f1 (+) origin of ss-DNA replication [pBluescript SK (+) only]	135–441
f1 (-) origin of ss-DNA replication [pBluescript SK (-) only]	21–327
β -galactosidase α -fragment coding sequence (<i>lacZ'</i>)	460–816
multiple cloning site	653–760
T7 promoter transcription initiation site	643
T3 promoter transcription initiation site	774
<i>lac</i> promoter	817–938
pUC origin of replication	1158–1825
ampicillin resistance (<i>bla</i>) ORF	1976–2833

(Fermentas)

Appendix 4

Maps of pNS2A-HAHis, pNS2B-HAHis, pNS4A-HAHis, and pNS4B-HAHis



(徐婕琳, 2003, 交大碩士論文)