

**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.

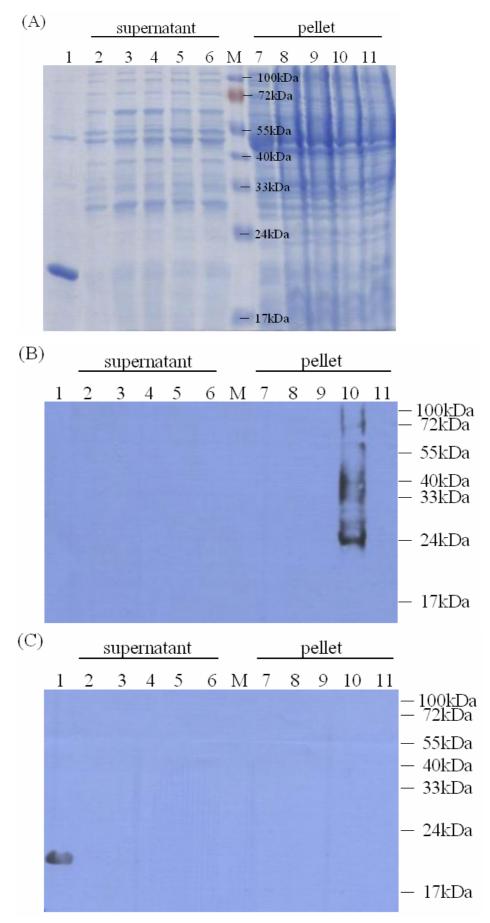
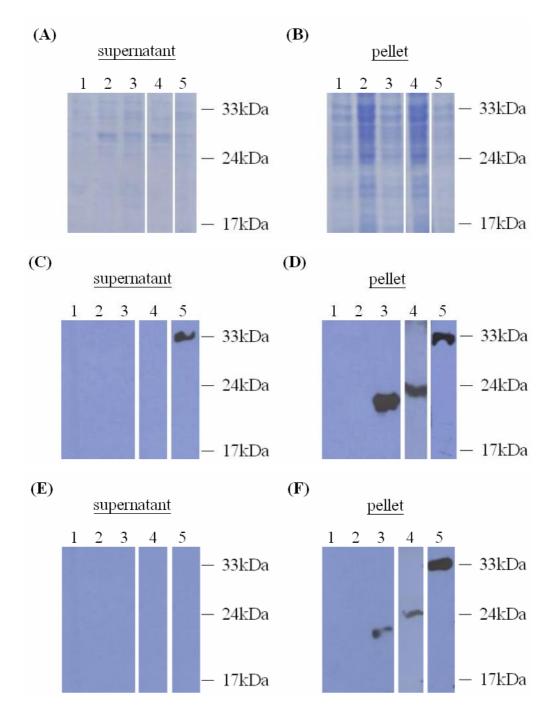


FIG. 3.15 *E. coli* NovaBlue(*DE3*) expressed nonstructural proteins analyzed by coomassie blue staining and Western blot. (A) Coomassie blue staining of the supernatant and pellet of NovaBlue(*DE3*) transformed with different plasmids. (B)

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, pETΔ5T-2A-HAHis; lane 3, pETΔ5T-2B-HAHis; lane 4, pETΔ5T-4A-HAHis; lane 5, pETΔ5T-4B-HAHis.

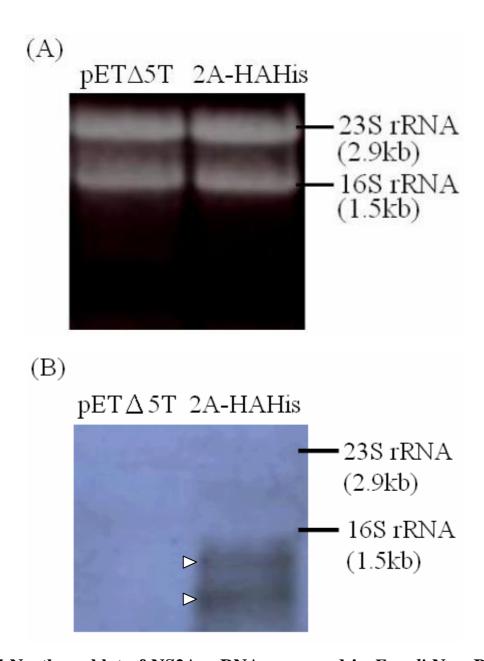
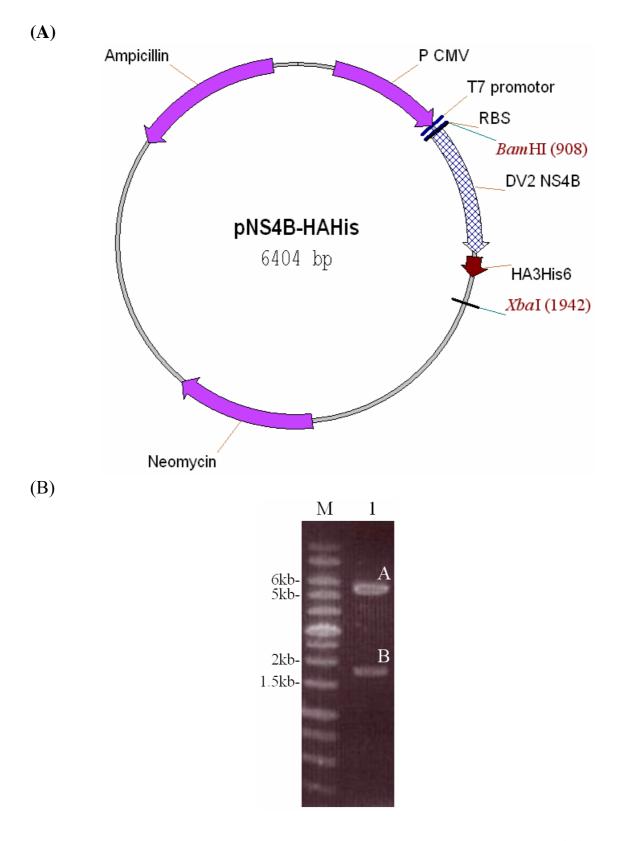


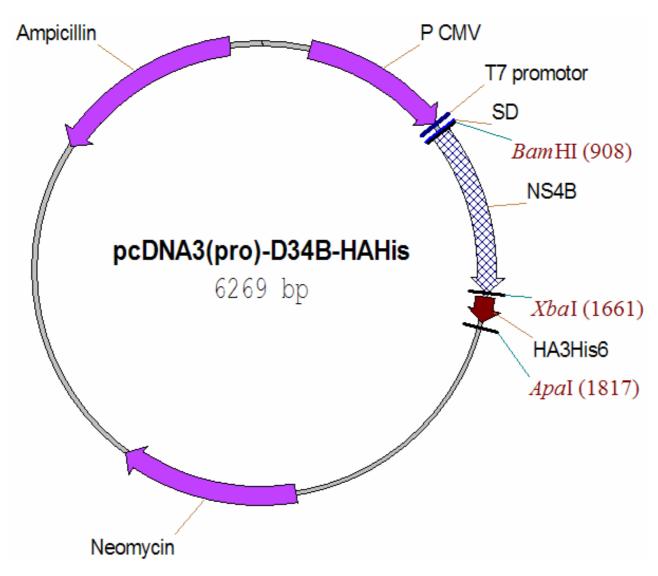
FIG. 3.17 Northern blot of NS2A mRNA expressed in *E. coli* NovaBlue(*DE3*). (A) RNA of NovaBlue(*DE3*) transformed with pET $\Delta$ 5T or pET $\Delta$ 5T-2A-HAHis before transfer. (B) Northern blot of mRNA expressed in NovaBlue(*DE3*) transformed with pET $\Delta$ 5T or pET $\Delta$ 5T-2A-HAHis.



**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



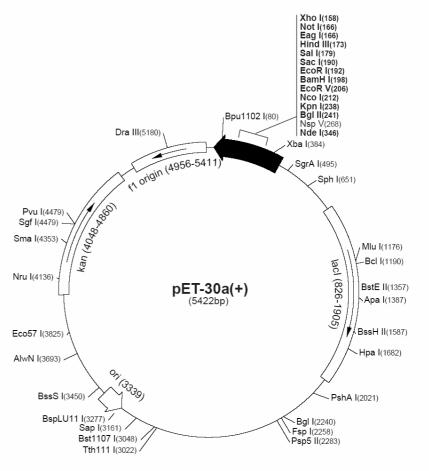
(From 陳欣悟, Yang laboratory)

# Appendix 2

### **Map of pET-30a(+)**

pET-30a(+) sequence landmarks	
T7 promoter	419-435
T7 transcription start	418
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites	
(Nco I - Xho I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	826-1905
pBR322 origin	3339
Kan coding sequence	4048-4860
f1 origin	4956-5411

The maps for pET-30b(+) and pET-30c(+) are the same as pET-30a(+) (shown) with the following exceptions: pET-30b(+) is a 5421bp plasmid; subtract 1bp from each site beyond BamH I at 198. pET-30c(+) is a 5423bp plasmid; add 1bp to each site beyond BamH I at 198.

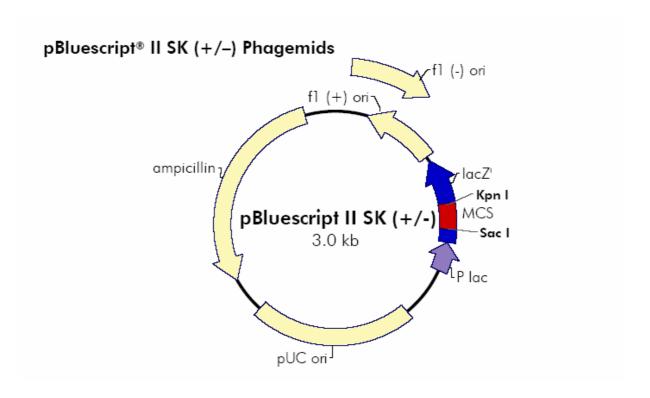


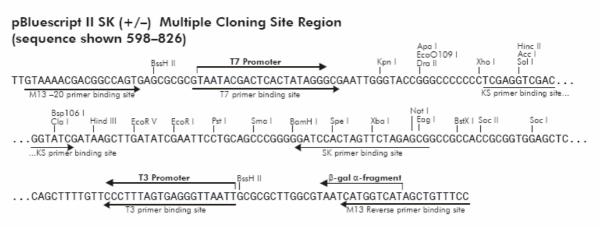
	T7 promoter primer #69348-3	3				
pET upstream primer #69214-3	T7 promoter	lac operator	Xba I	rbs		
AGATCGATCTCGATCCCGCGAA	7,001					
Nde l His∙Tag			S•Ta	g <u>Nsp∨</u>	Bgl II	
TATACATATGCACCATCATCAT MetHisHisHisHis	TATACATATGCACCATCATCATCATCATCTCTGGTCTGG					
Kpn I pET-30a(+)	Ncol EcoRV BamHIE	"thrombin <u>EcoRI SacI Sall Hind</u>	Eag I I III <u>Not I Xho</u>	His∙Tag		
GGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACCAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA GlyThr <u>AspAspAspAspLys</u> AlaMetAlaAspIleGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProPeuArgSerGlyCysEnd						
enterokinase '						
pET-30b(+)	pET-30b(+)GCGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCACTCGAGCACCACCACCACCACCACCACCACCACCACCACCAC					
pET-30c(+)	pET-30c(+)GGATATCTGTGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACCACCACGAGATCCGGCTGCTAAGlyTyrLeuTrp!leArg!leArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrThrGlu!leArgLeuLeu					
	<i>Bpu</i> 1102	?1	T7 terminator			
CAAAGCCCGAAAGGAAGCTGAG	CAAAGCCCGAAAGGAAGCTGAGTTGGCTGCCACC <mark>G</mark> GTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG					
	T7 terminator primer #69337-3					
pET-30a-c(+) cloning/expression region						

(Novagen)

## Appendix 3

#### Map of pBluescript II SK (+)



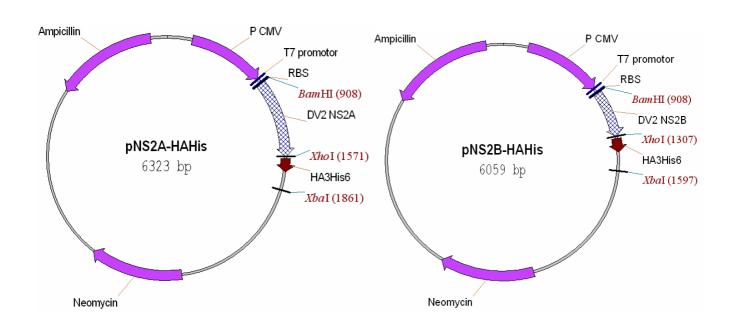


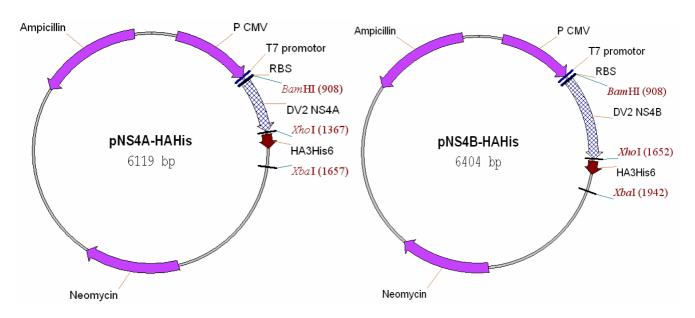
Feature	Nucleotide Position
f1 (+) origin of ss-DNA replication [pBluescript SK (+) only]	135–441
f1 (–) origin of ss-DNA replication [pBluescript SK (–) only]	21–327
β-galactosidase α-fragment coding sequence (lacZ')	460-816
multiple cloning site	653–760
T7 promoter transcription initiation site	643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