

(19)
(12)(KR)
(A)(51) 。 Int. Cl.⁷
C12N 1/21(11)
(43)10-2004-0081237
2004 09 21(21) 10-2003-0015965
(22) 2003 03 14

(71) 373-1

(72) 212-702

373-1

1 149-31

(74)

:

(54)

(inclusion body - associated protein)

(*ibpA* / *ibpB*)

가 . , *ibpA* / *ibpB* 가 (*ibpA* / *ibpB*)
 . , *ibpA* / *ibpB* 가

4

, *ibpA*, *ibpB*,

1 pSK1bpABKm . *ibpA'* *ibpA* 5' 500 bp, *ibpB'* *ibp*
B 3'

2	pACTacIbpA	.			
3	pACTacIbpB	.			
4	pACTacIbpAB	.			
5	pACIbpAB	.			
6	pTac99GFP	.			
7	p223-3IFN- γ	.			
8	pTac99IL-12p40	.			
9	pTrcS1PhoA SDS-PAGE		W3110		4
10	pTrcS1PhoA SDS-PAGE		WIB101		4
11	pTrcSO _b 4 SDS-PAGE		W3110	WIB101	4
12	p184 Cm, pACTacIbpA, pACTacIbpB, pACTacIbpAB W3110 WIB101 SDS-PAGE				pYKM-I ₁
13	p184 Cm, pACTacIbpAB, pACIbpAB W3110 WIB101 SDS-PAGE				pYKM-I ₁
14	p184 Cm, pACTacIbpAB, pACIbpAB W3110 WIB101 SDS-PAGE				p223-3IFN- γ
15	p184 Cm, pACTacIbpAB, pACIbpAB W3110 WIB101 SDS-PAGE				pTac99IL-12p40
16	p184 Cm, pACTacIbpAB, pACIbpAB W3110 WIB101 SDS-PAGE				pTac99GFP
17	p184 Cm, pACTacIbpAB W3110 SDS-PAGE				pTac99GFP

(inclusion body-associated protein)

(*ibpA* / *ibpB*)
ibpA / *ibpB* 가
 , *ibpA* / *ibpB*

가

DNA

(molecular chaperones)

(Heat Shock Proteins: HSPs)

HSPs SecB, DnaK, DnaJ, GrpE, GroEL, GroES, IbpA, IbpB () DNA
 , RNA , (folding), (recycling), (: L
 aRossa and Van Dyk, Mol. Microbiol. 5, 529-534, 1991). 가 HSPs DnaK-Dna
 J-GrpE GroEL-GroES DnaK 가 , GrpE
 DnaK가 (native protein) (folding)
 GroEL-GroES 가

HSPs

가

가

HSPs . Goloubi
 noff GroEL GroES (ribulose biphosphate carboxylases; Rubiscos
) (: Goloubinoff et al., Nature 337, 44-47, 1989). , Georgiou V
 alax GroEL GroES DnaK DnaJ - (β-galactosidase) (:
 Georgiou and Valax, Curr. Opin. Biotechnol. 7, 190-197, 1996). Murby
 (secretion) DnaK (alkaline phosphatase)
 (fusion proteins) (Murby et al., Biotechnol. Appl. Biochem. 14, 33
 6-346, 1991). 가 가 : (1) HSPs
 (30-50%)
 가 HSPs (capacity) ; (2) (folding)
 (stability) ; (3) 가 (Langer et al., Nature 356, 6
 83-689, 1992). HSPs
 . Baneyx DnaK 2-4 가 (: US 5,5
 52,301).

, HSPs (*rpoH*)가
 . *rpoH* (32; δ³²)가 (folding) (degradation)
 HSPs (protease)
 . Easton *rpoH*
 2 (bovine insulin-like growth factor 2; BIGF2) 20-25% (: Easton et al., Gene 10
 1, 291-295, 1991). Obukowicz *rpoH* (:
 : Obukowicz et al., Appl. Environ. Microbiol. 58, 1511-1523, 1992). , Goldberg *rpoH* *lon*
 (: US 4,758,512).

(small heat shock proteins: sHSPs)

sHSPs

(15-30 kDa) 가 HSPs

. sHSPs

(eukaryotes)

(prokaryotes)

(Human) sHSPs (HSP27, αA-, αB-crystallin), murine
 HSP25, (*Pisum sativum* (pea)) HSP18.1, (*Saccharomyces cerevi*
siae) HSP26, (*Bradyrhizobium japonicum*) sHSPs (HSPH, HSPB, H
 SPC, HSPF), (*Methanococcus jannaschii*) HSP16.5, (*Sy*
nechococcus vulcanus) HSP16, (*Mycobacterium tuberculosis*) H
 SP16.3 (: Studer and Narberhaus, J. Biol. Chem. 275, 37212-37218, 2000). ,
 sHSPs IbpA / IbpB가
 (: Allen et al., J. Bacteriol. 174, 6938-6947, 1992). , sHSPs IbpA /
 IbpB ()

IbpA / IbpB가 (periplasm) : (1)

(: Nossal et al., J. Biol. Chem., 241, 3055-3062, 1966), (2)

(: Meerman and Georgiou, Ann. N. Y. Acad. Sci., 721, 292-302, 1994), (3)

(folding) (: Hockney, TIBTECH, 12, 456-463, 1994).

ibpA / *ibpB*

pB

(: Makrides SC, Microbiol. Rev. 60, 512-538, 1996).

(1)

(: Lee SY, Trends Biotechnol. 14, 98-105, 1996); (2)

80-98%

1-30% (: Jeong and Lee, Appl. Environ. Microbiol. 65, 3027-3032, 1999); (3)

(: Rudolph R., Wiley, New York, 1995; Kane and Hartley, Trends Biotechnol., 6, 95-101, 1988). (solubilization) (renaturation)

(: Deneffe et al., Gene, 56, 61-70, 1987; Saito et al., J. Biochem., 101, 1281-1288, 1987; Gill et al., Bio/Technol., 3, 643-646, 1985; Weir and Sparks, Biochem. J., 245, 85-91, 1987).

(inclusion body-associated protein) (*ibpA* / *ibpB*)

B)가 (inclusion body-associated protein) (*ibpA* / *ibpB*)

ibpA / *ibpB* 가

ibpA / *ibpB* 가

ibpA / *ibpB* 가

ibpA / *ibpB* 가

ibpA / *ibpB* 가

(alkaline phosphatase) (leptin)

ibpA / *ibpB* 가 pACTac

IbpAB, pACIbpAB, pACTacIbpA *ibpA* / *ibpB* pACTacIbpB

ibpA / *ibpB*
pACTacIbpAB, pACIbpAB, pACTacIbpA pACTacIbpB

ibpA / *ibpB*

ibpA / *ibpB*

ibpA / *ibpB*

(inclusion body)

ibpA / *ibpB*

-I (insulin-like growth factor-I), 12 (interleukin 12 β chain), (interfe
ron- γ), (green fluorescent protein; GFP)

ibpA / *ibpB*

(inclusion body-associated protein)

(*ibpA* /

ibpB)가

(inclusion body-associated protein)

(*ibpA* / *ibpB*)

ibpA /

ibpB

ibpA / *ibpB* 가
ibpA / *ibpB* 가

가

가

가

1: *ibpAB* 가

W3110 (ATCC 39936) DNA (Sambrook) (: Sam
brook et al. Molecular Cloning, 2nd ed, Cold Spring Harbor Laboratory Press, NY, 1989). W3110 50
0 mL LB (Luria-Bertani medium) 24
, 10 mg/mL (lysozyme, Sigma Co., USA) TE
(10 mM Tris, 1 mM EDTA; pH 7.6) 50 mL 24
10% (sodium dod
ecyl sulfate, SDS) 16 mL 20 mg/mL K (Proteinase K, Sigma Co., USA) 570 μ l 가
, 37 1 , 5 M 14 mL 0.7 M
10% (cetyltrimethylammoniumbromide, CTAB, Sigma Co., USA) 10.66 mL
가 , 65 10 , 2 6,000 rpm
10 (chl
oroform:isoamylalcohol = 24:1) 가 , 2 DNA
DNA , 1 mL TE
DNA DNA RNase (Sigma Co., USA) 50 μ g/mL 가 , 37
1 (c
hloroform:isoamylalcohol = 24:1) 가 , 2 6,000 rp
m 10 가 DNA
1 mL TE DNA W3110 DNA

ibpAB λ *red* (operon) (: Jeong and Lee, Appl. Environ. Microbiol. 68, 4979-4985, 2002). (homologous recombination) pTrcEBG W3110

1 mM IPTG 가 W3110(pTrcEBG) *red* (electroporation-competent cell)

W3110 DNA 1 2 *i*

bpAB 3' 500 bp , 3 4 *ibpAB*

Not I *Xba* I , *Eco* R I *Sal* I , 5 6

600 bp pSKlbpABKm (1). pSKlbpABK

Xba I *Eco* R I 1 4 PCR WIB101 . WI

B101 *ibpAB* . *ibpAB* 가 *ibpAB* 가 (polymerase chain reaction: PCR)

5 6 (denaturation) 95 5 1 , 95 5

0 , (annealing) 55 1 , (extention) 72 1 30 , 30

72 5 1 .

5'-ataagaatgcggccgcccagctgtggatcaccgaaactgat-3' (1)

5'-gctctagatgcatagactgagggggcagca-3' (2)

5'-ggaattcttcgactgtttaagatatttcgg-3' (3)

5'-acgcgtcgacggagaaaatccccagcactaccgg-3' (4)

5'-gctctagagccacgttgtgtctcaaa-3' (5)

5'-cgaattcttagaaaaactcatcgagca-3' (6)

2: *ibpA* / *ibpB*

IbpA IbpB

W3110 DNA , 7 8, 9 10, 11 12

1 PCR *ibpA* , *ibpB* , *ibpAB* . *ibpA* , *ibpB* , *ib*

pAB *Eco* RI *Hin* dIII pTac99A pTac99I

bpA, pTac99IbpB, pTac99IbpAB pTac99A pTAc99A (Pharmacia Biotech., Uppsala, Sweden) *trc* pKK223-3 (Pharmacia Biotech., Uppsala, Sweden) *tac*

. pKK223-3 *tac* *Pvu* II *Eco* RI *tac*

pTrc99A pTac99IbpA, pTac99I

bpB, pTac99IbpAB *Ssp* I *Dra* I *Pvu* II pACYC184 (Ne

w England Biolabs, USA) pACTacIbpA, pACTacIbpB, pACTacIbpAB

(2, 3, 4).

ibpAB W3110 DNA ,

13 14 1 PCR *ibpAB* *ibpAB*

PCR *Eco* RI pACIbpAB (5). pACYC184 (Ne

w England Biolabs, USA)

5'-ggaattcatgcgtaactttgatttatccc-3' (7)

5'-cccaagcttttagttgatttcgatacggcgc-3' (8)

5'-ggaattcatgcgtaacttcgatttatccccactg-3' (9)

5'-cccaagcttttagctatttaacgcgggacgttcgct-3' (10)

5'-ggaattcatgcgtaactttgatttatccc-3' (11)

5'-cccaagcttttagctatttaacgcgggacgttcgct-3' (12)

5'-ggaattccagctgtggatcaccgaaactg-3' (13)

5'-ggaattcagaacgtgccgaaatatctta-3' (14)

3:

(*Aequorea victoria*) (green fluorescent protein: GFP)
 15, *gfp_{uv}* 16 pGFP_{uv} (Stratagene Cloning Systems, USA)
 GFP PCR . PCR 1 pTac99A pTa
 c99GFP (*Eco* RI *Hin* dIII) (6).

(interferon- γ : INF- γ) (Cytokine) (http://c
 ytokine.chonbuk.ac.kr/) pUC18/INF- γ
 17, 18 PCR . PCR 1
 INF- γ *Eco* RI *Hin* dIII p223-3INF- γ pKK223-
 3 (Pharmacia Biotech., Uppsala, Sweden) (7).

12 (interleukin 12 β chain: IL-12p40)
 (http://cytokine.chonbuk.ac.kr/) pUC18
 /p40 , 19, 20 PCR . PCR 1
 IL-12p40 *Eco* RI *Hin* dIII
 pTac99A pTac99IL-12p40 (8).

5'-ggaattcatgagtaaaggagaagaacttt-3' (15)

5'-cccaagcttttatttgatgagctcatcc-3' (16)

5'-ggaattcatgtgttactgccaggacccatat-3' (17)

5'-cccaagcttttactgggatgctcttcgacc-3' (18)

4: *ibpAB* 가

pTrcS1PhoA (: Choi et al., Appl. Microbiol. Biotechnol. 53, 6
 40-645, 2000) 1 WIB101 W3110
 가 LB
 250 mL 37 , LB (100 mL)
 600 nm (O.D.)가 0.7 0.01 mM
 IPTG (isopropyl- β -thiogalactoside) 가
 1 mL ,
 0.1 mL ,
 L 4 6000 rpm 5 0.5 mL TE 1 m
 6000 rpm 5 0.2 mL TE 4
 4 10,000 rpm 10 ,
 ,
 1 mL 4 6000 rpm 5
 30 mM Tris-HCl (pH 8.0) 0.4 mL , 1 m
 M EDTA 가 10 4 10,000 rpm 10
 , 4 10,000 rpm 10 0.4 mL 10
 , 0.2 mL TE

(: Ausubel et al., Current Protocols in Molecular Biology, 1989).

200 $\mu\ell$ SDS-PAGE (25% , 2% SDS, 14.4 mM 2-
(bromophenol blue), 60 mM Tris-HCl) 50 $\mu\ell$
, 0.1% (separating gel) SDS-PAGE (40%,
12% 10%, 0.25 g/L R (Coomassie brilliant blue R)) 2 가
(40% , 7%) 2 가 (9).

9 pTrcS1PhoA W3110 SDS-PAGE
5 4 9 M , 1
($\leftarrow$)

9 W3110 50 kDa
SDS-PAGE
가 20%

10 pTrcS1PhoA WIB101 SDS-PA
GE 1 5 4 10 M ,
($\leftarrow$)

10 WIB101 50 kD
a SDS-PAGE
가 30%

(: Brickman and Beckwith, J. Mol. Bi
ol., 96, 307-316, 1975).
50 mM Tris-HCl(pH 7.5) 1 mL ,
0.1 mL 가 37 5 , 0.4% PNPP (p-nitrophenyl phosphate) 0.1 mL
가 37 5 , 1M K₂HPO₄ 0.1 mL 12,000 rpm
5 50 mM Tris-HCl 420 n
m 550 nm (U/mL)

활성도 = 1000 × $\frac{(Abs_{420nm} - 1.75 \times Abs_{550nm})}{[반응시간(분) \times 반응부피(mL) \times 세포광학밀도(O.D.)]}$

pTrcS1PhoA W3110, WIB101

[1]

		(U/mL)
W3110		0.04
pTrcS1PhoA	W3110	1405
WIB101		0.05
pTrcS1PhoA	WIB101	4720

1 pTrcS1PhoA WIB101
pTrcS1PhoA W3110 3 가
가
W3110 가
01 WIB1
01 가 W3110 2 WIB1
6 *ibpAB* 가 WIB101

5: *ibpAB* 가 (leptin)

1 pTrcSOB4 (: Jeong and Lee., Biotechnol. Bioeng. 67, 398-407, 2000)
WIB101 W3110 4
600 nm (O.D.)가 0.7 1 m
M IPTG (isopropyl- β -thiogalactoside) 가 4
1 mL 4
(11).

11 pTrcSOB4 W3110 WIB101 S
DS-PAGE 11 , M
1 3 4 W3110
4 6 4
WIB101 , (←)

11 W3110 50%가
WIB101
WIB101 가 W3110 3
3 *ibpAB* 가 WIB101

6: *IbpA* / *IbpB* -I (human insulin-I) like growth factor-I: IGF-I)

2 (p184 Cm, pACTacIbpA, pACTacIbpB, pACTacIbpAB, pA
ClbpAB) -I pYKM-I₁ (: Kim and Lee, J. Biotechnol. 48, 9
7-105, 1996) 1 WIB101 W3110
4 -I
600 nm (O.D.)가 0.7 1mM IPTG (isopropyl- β -thiogalactoside) 가
-I 4
-I 4
(12, 13).

12 p184 Cm, pACTacIbpA, pACTacIbpB, pACTacIbpAB pYKM-I₁
W3110 WIB101 SDS-PAGE
12 , M 1 p184 Cm pY
W3110, 2 pACTacIbpAB pYKM-I₁ W3110, 4
3 pACTacIbpA pYKM-I₁ W3110, 5 p184 Cm
pACTacIbpB pYKM-I₁ W3110, 6 pACTacIbpAB pYKM-I₁ WIB101, 8
pYKM-I₁ WIB101, 7 pACTacIbpA pYKM-I₁ WIB101
, (←) -I

13 p184 Cm, pACTacIbpAB, pACIbpAB pYKM-I₁
 W3110 WIB101 SDS-PAGE
 13 , M , 1 p184 Cm pYKM-I₁ WIB101,
 W3110, 2 p184 Cm pYKM-I₁ W3110, 4 pA
 3 pACTacIbpAB pYKM-I₁ WIB101, 5 pACIbpAB pYKM-I₁ WIB101
 CTacIbpAB pYKM-I₁ W3110, 6 (←) -I .

12 13 W3110 *ibpA*, *ibpB* *ibpAB*
 -I (p184 Cm pYKM-I₁ W3110) 2 가
 , *ibpAB* 가 WIB101
 lbpA lbpB .
 , *ibpA*, *ibpB* *ibpAB* 가
 ACTac *ibpAB* pAC *ibpAB*) 가 . *ibpAB* (p

7: lbpAB(interferon- γ : IFN- γ)

2 (p184 Cm, pACTacIbpA, pACIbpAB) 3
 W3110 p223-3IFN- γ 1 WIB101,
 600 nm 4 (O.D.)가 0.7 1 mM IPTG (isopro
 pyl- β -thiogalactoside) 가 4
 (14).

14 p184 Cm, pACTacIbpAB, pACIbpAB p223-3IFN- γ
 W3110 WIB101 SDS-PAGE
 14 M , 1 p184 Cm p223-3IFN- γ WIB10
 W3110, 2 p184 Cm p223-3IFN- γ W3110, 4
 1, 3 pACTacIbpAB p223-3IFN- γ WIB101, 5 pACIbpAB
 p223-3IFN- γ W3110, 6 pACIbpAB p223-3IFN- γ
 WIB101 (←)

14 W3110 lbpAB
 (p184 Cm p223-3IFN- γ W3110) 2 가 ,
 lbpAB가 WIB101
 lbpA lbpB .

8: lbpAB**12 (interleukin 12 β chain)**

2 (p184 Cm, pACTacIbpA, pACIbpAB) 3
 12 pTac99IL-12p40 1 WIB101,
 W3110 4
 12 600 nm (O.D.)가 0.7 1 mM IP
 TG (isopropyl- β -thiogalactoside) 가 12
 4 12
 4 (15).

15 p184 Cm, pACTacIbpAB, pACIbpAB pTac99IL-12p40
 W3110 WIB101 SDS-PAGE
 15 M , 1 p184 Cm pTac99IL-
 12p40 W3110, 2 p184 Cm pTac99IL-12p40

WIB101, 3 pACTacIbpAB pTac99IL-12p40 W3110, WIB101, 5 W3110,
 4 pACIbpAB pTac99IL-12p40 pTac99IL-12p40 W3110, 6 WIB101, 5 pACIbpAB pTa
 c99IL-12p40 WIB101 (←)

15 W3110 lbpAB 12
 (p184 Cm pTac99IL-12p40 W3110) 1.5 가
 , lbpA lbpB WIB101

9: lbpAB (*A. victoria*) (green fluorescent protein: GFP)

2 (p184 Cm, pACTacIbpA, pACIbpAB) 3
 (GFP) pTac99GFP 1
 WIB101, W3110 4
 600 nm (O.D.)가 0.7 1 mM IPTG (isopro
 pyl-β-thiogalactoside) 가 4
 (16, 17).

16 p184 Cm, pACTacIbpAB, pACIbpAB pTac99GFP
 W3110 WIB101 SDS-PAGE
 16 M , 1 p184 Cm pTac99GFP p184 Cm pTac99GFP
 W3110, 2 WIB101, 4 WIB101,
 3 ACTacIbpAB pTac99GFP pTac99GFP W3110, 4 pACIbpAB pTac99GF
 P W3110, 6 pACIbpAB pTac99GFP WIB1
 01 (←)

16 W3110 lbpAB (p184 C
 m pTac99GFP W3110) 2 가
 lbpAB가 WIB101 lbpA lbpB

17 p184 Cm, pACTacIbpAB pTac99GFP 17
 W3110 SDS-PAGE 1 3 p184 Cm pTac99GFP
 M , 4 6 pACTacIbpAB
 W3110 ,
 pTac99GFP W3110 (←)

17 W3110 lbpAB 100%
 가

(inclusion body-associated protein) (*ibpA* /
ibpB) 가 , *ibpA* / *ibpB* 가

, *ibpA* / *ibpB* 가

가 ,

(57)

1. *ibpA* / *ibpB* 가 .
2. 1 , .
3. 2 , *ibpAB* 가 WIB101.
4. 1 2 , .
5. 4 , 가 .
6. , 4 .
7. 6 , (leptin) (alkaline phosphatase) .
8. 6 , 가 .
9. *ibpA* / *ibpB* 가 pACTacIbpAB, pACIbpAB, pACTacIbpA pACTacIbpB.
10. *ibpA* / *ibpB* .
11. 10 , .
12. 10 11 , 9 *ibpA* / *ibpB* .
13. 10 11 , .
14. (inclusion body) , 13 .
15. 14 , .
- 16.

14 ,

17.

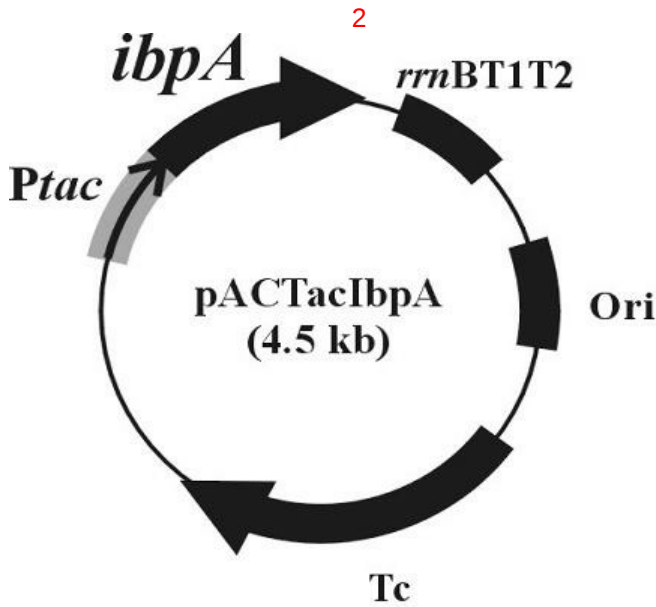
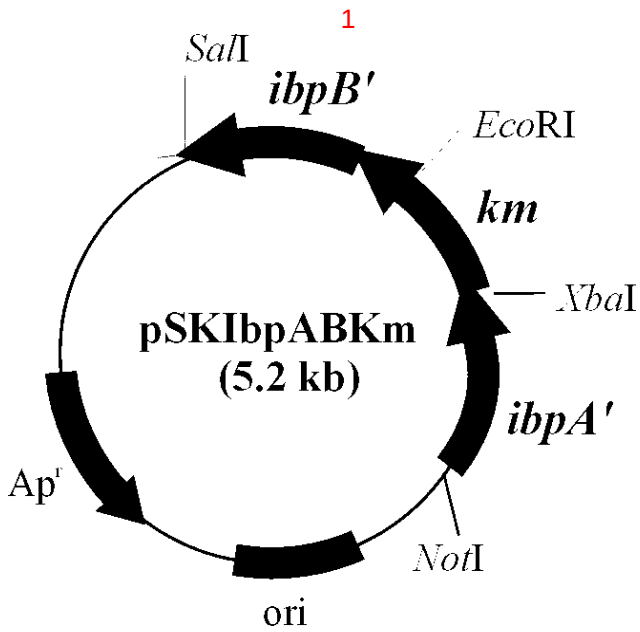
14 ,
(interleukin 12 β chain),
GFP)

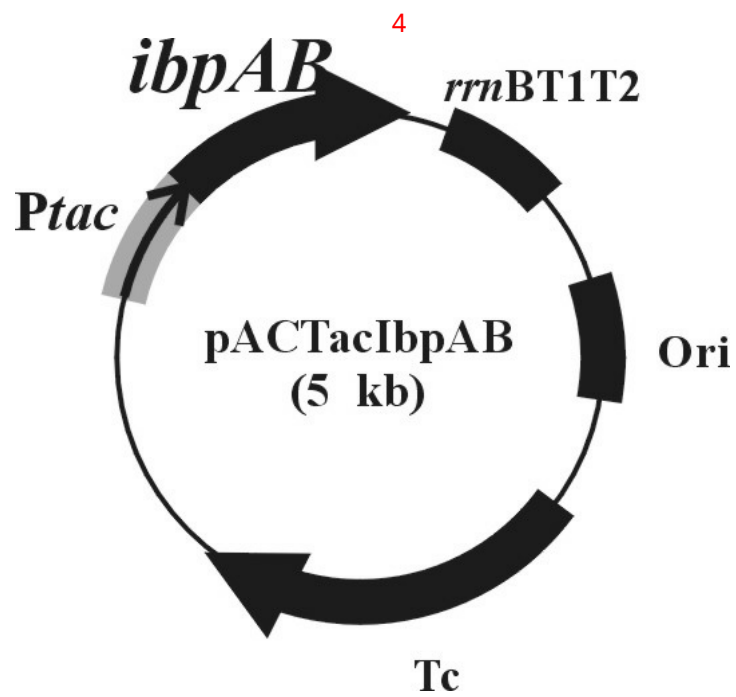
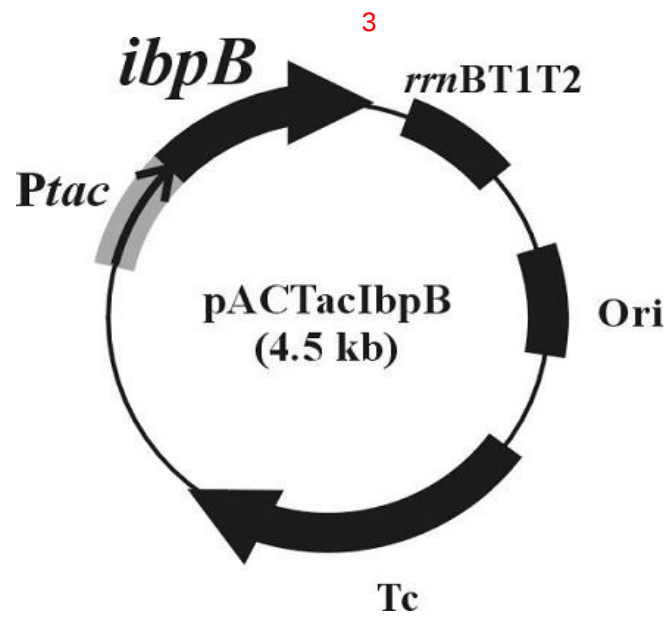
-I (insulin-like growth factor-I),
(interferon- γ),

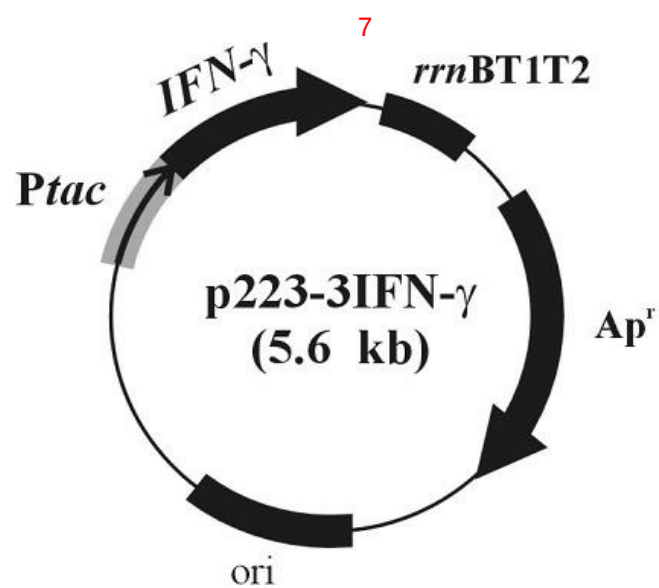
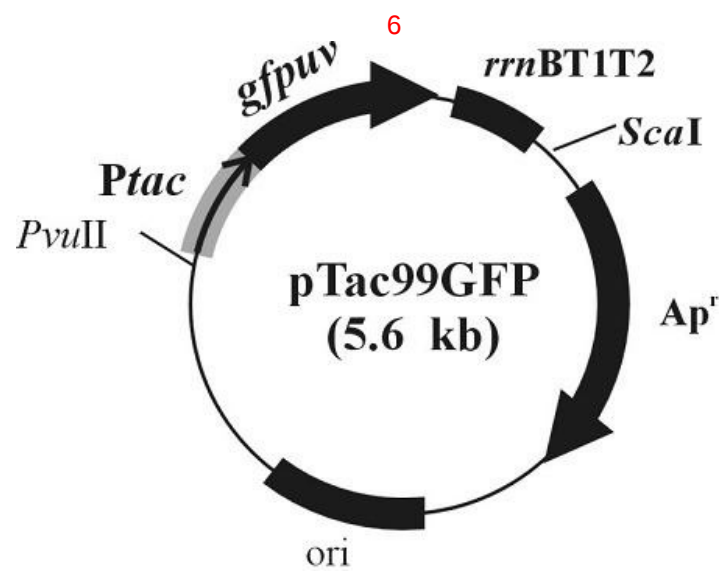
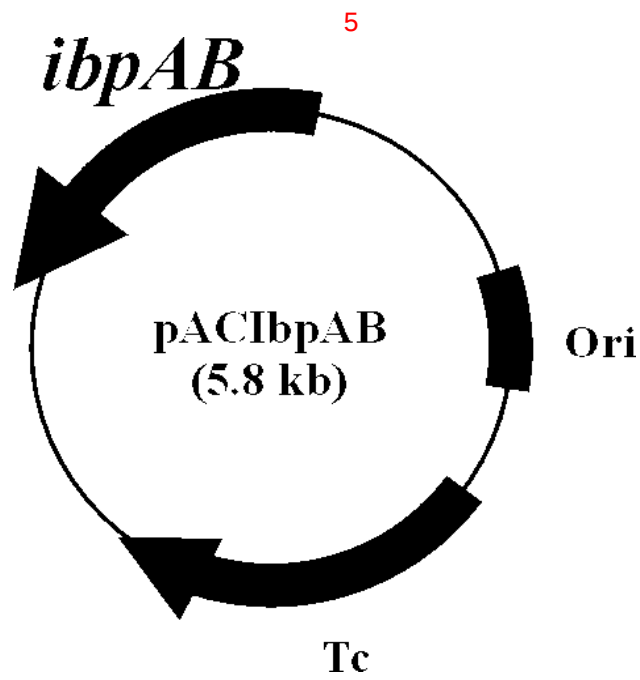
12
(green fluorescent protein;

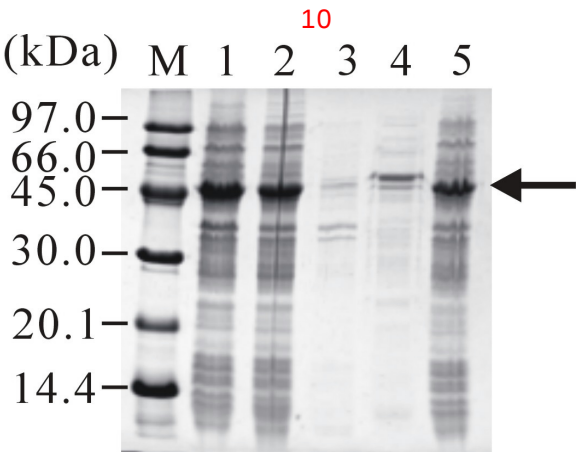
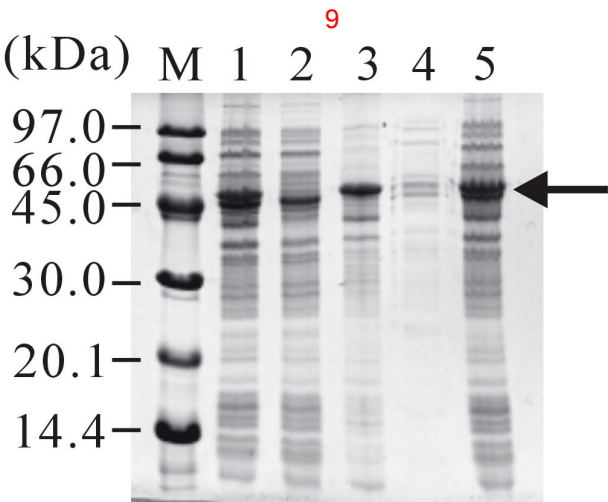
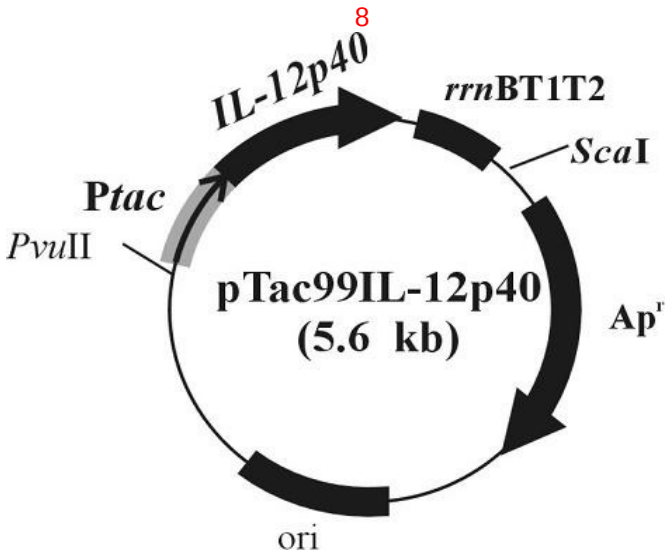
18.

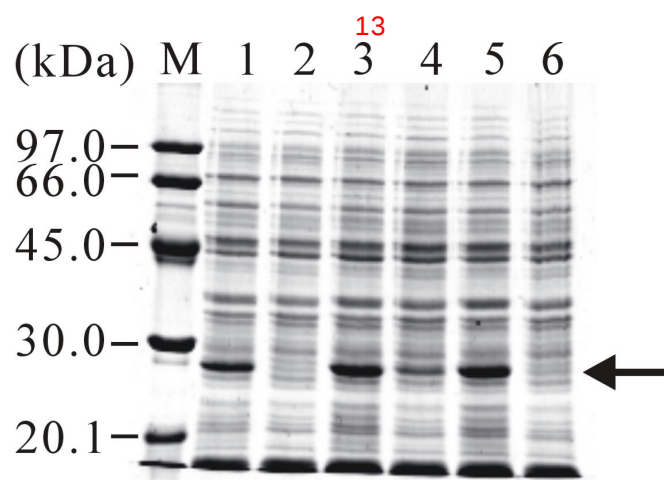
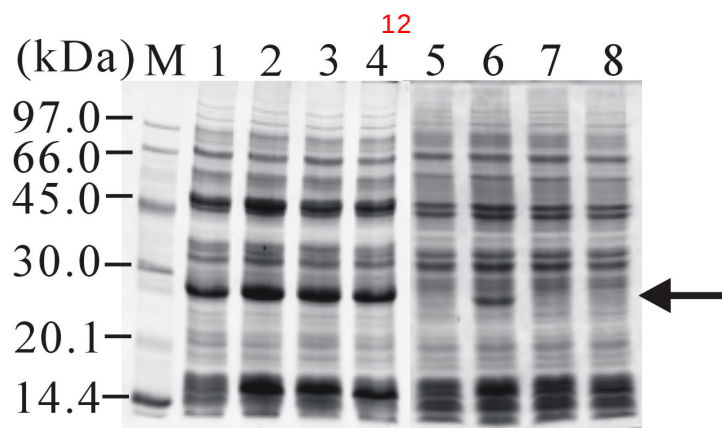
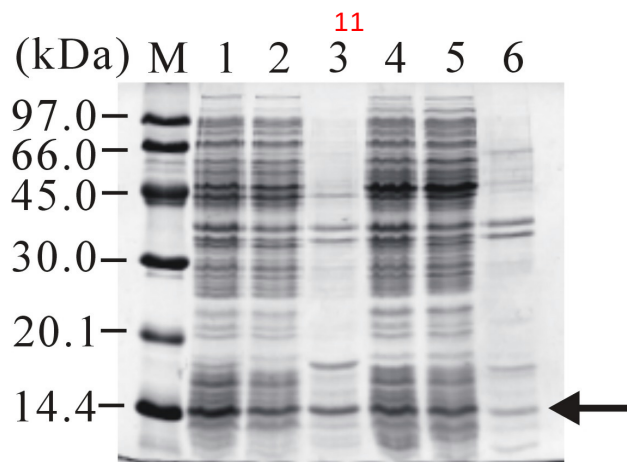
ibpA / *ibpB*

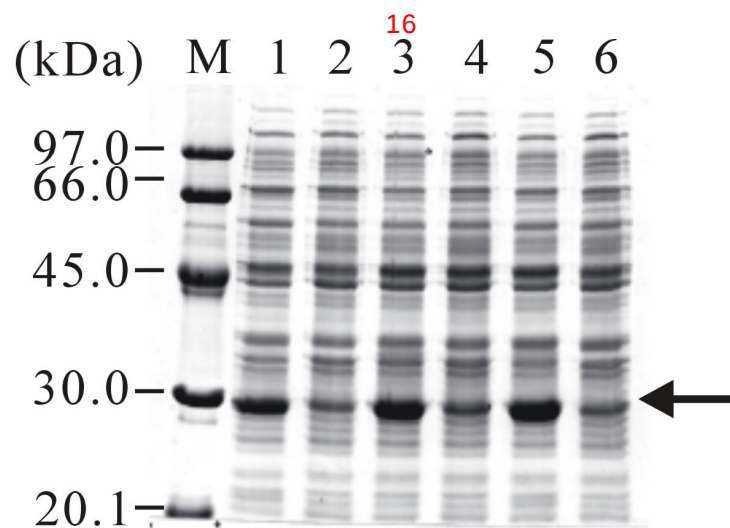
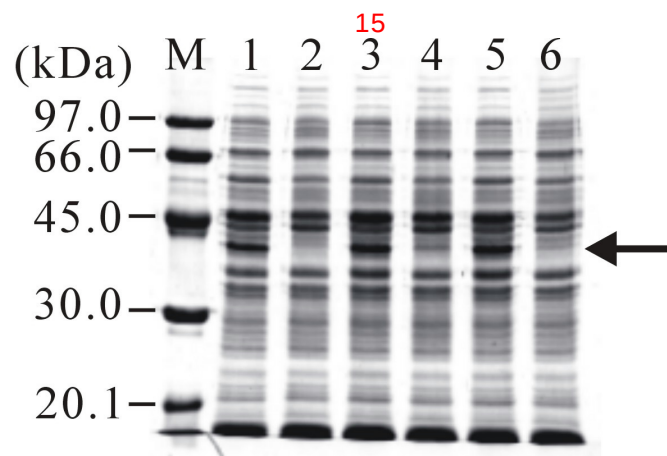
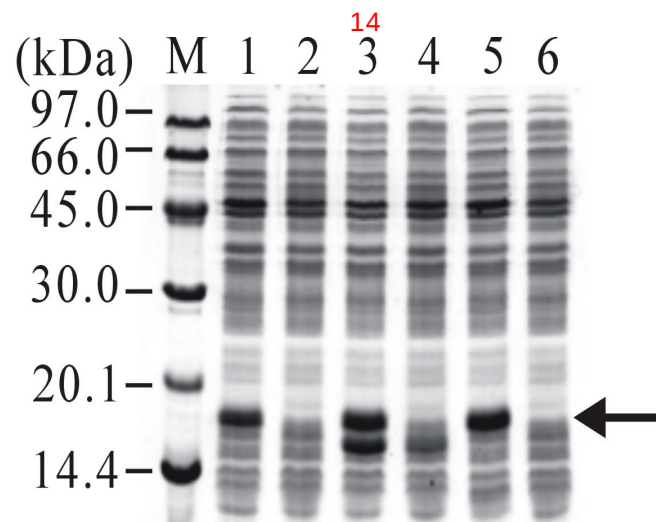


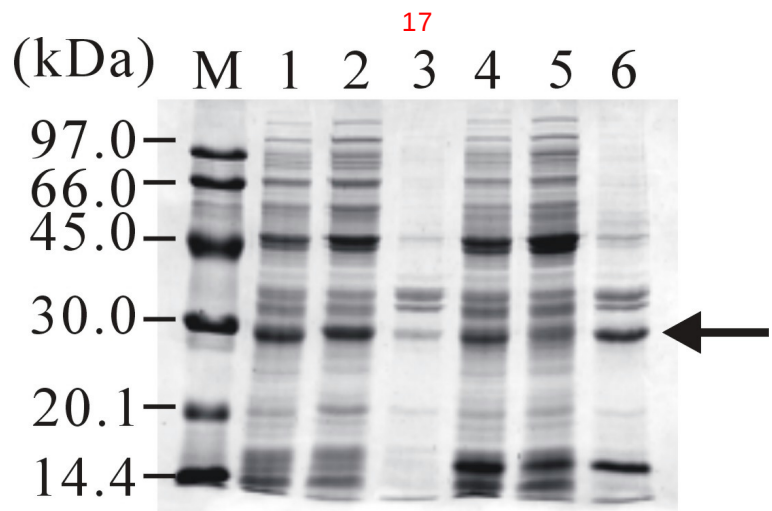












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