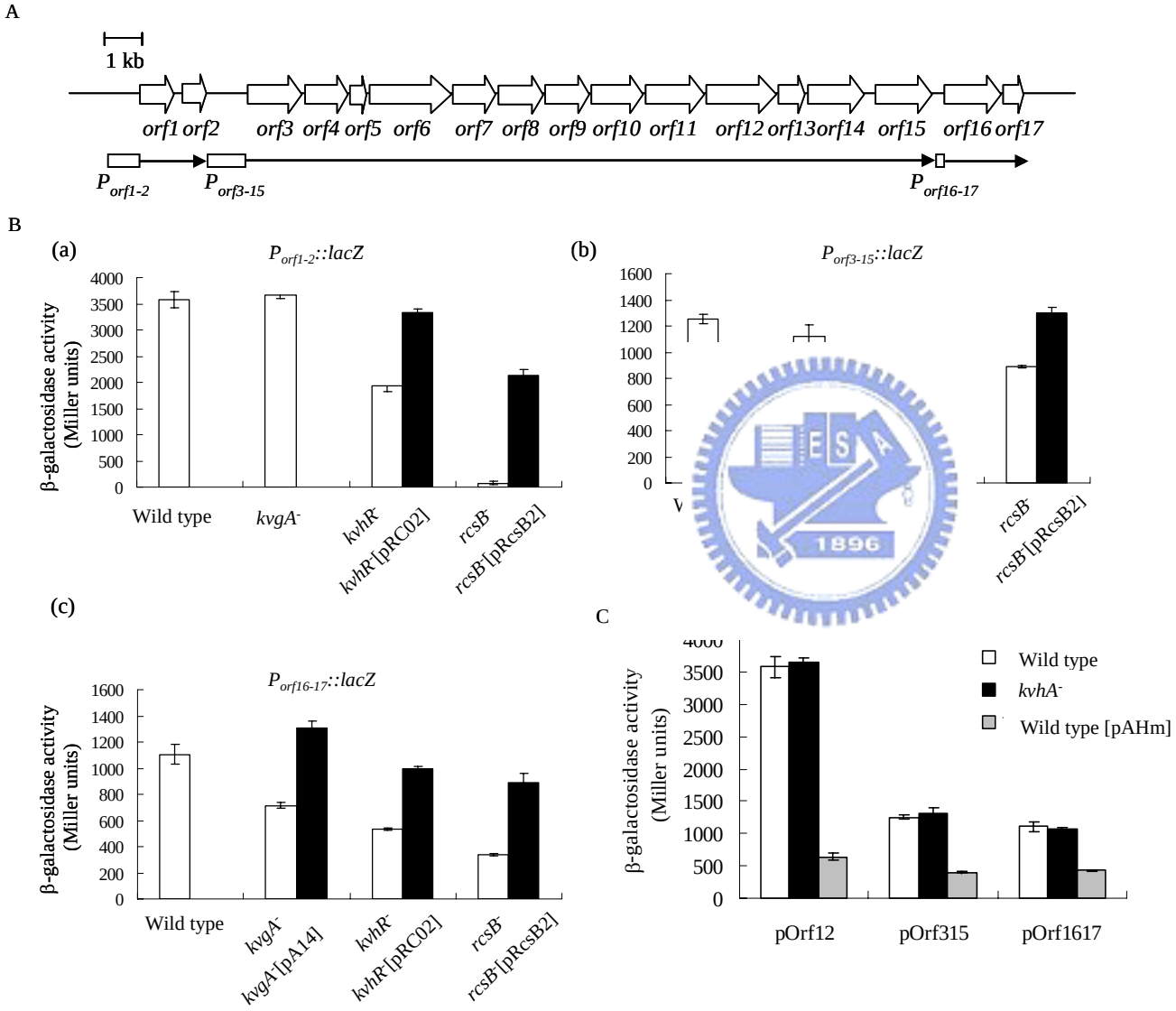

A
1 kb
orf1 orf2 orf3 orf4 orf5 orf6 orf7 orf8 orf9 orf10 orf11 orf12 orf13 orf14 orf15 orf16 orf17
P orf1-2
P orf3-15
P orf16-17
B
(a)
P orf1-2::lacZ
β-galactosidase activity (Miller units)
Wild type
kvgA-
kvhR-
kvhR-[pRC02]
rcsB-
rcsB-[pRcsB2]
(b)
P orf3-15::lacZ
rcsB-
rcsB-[pRcsB2]
(c)
P orf16-17::lacZ
kvgA-[pA14]
C
Wild type
kvhA-
Wild type [pAHm]
pOrf12
pOrf315
pOrf1617

Fig. 4. A. Organization of the *K. pneumoniae* K2 *cps* ge noters of the three *cps* transcripts are also indicated. The horizontal arrows that begin with a solid circle represent al units. **B.** Expression of K2 *cps* gene in various genetic backgrounds. The plasmids carrying $P_{orf1\text{-}2}$ (a), $P_{orf3\text{-}15}$ (b), and $P_{orf16\text{-}17}$ (c) promoter fused with *lacZ* gene and transferred into wild type, *kvgA*-, *kvhR*-, and *rcsB*- respectively by conjugation and shown as open bar. The complementation test was performed and shown as black bar. **C.** The plasmids, pOrf12, pOrf315, and pOrf1617, were transferred into wild type (open bar), *kvhA*- (black bar), and wild type strain carrying pAHm (gray bar). The *cps*-promoter carrying cells were grown in M9 medium to an OD600 of 0.7 and the β-galactosidase activities were measured and presented in Miller units as described in Materials and Methods. Methods.

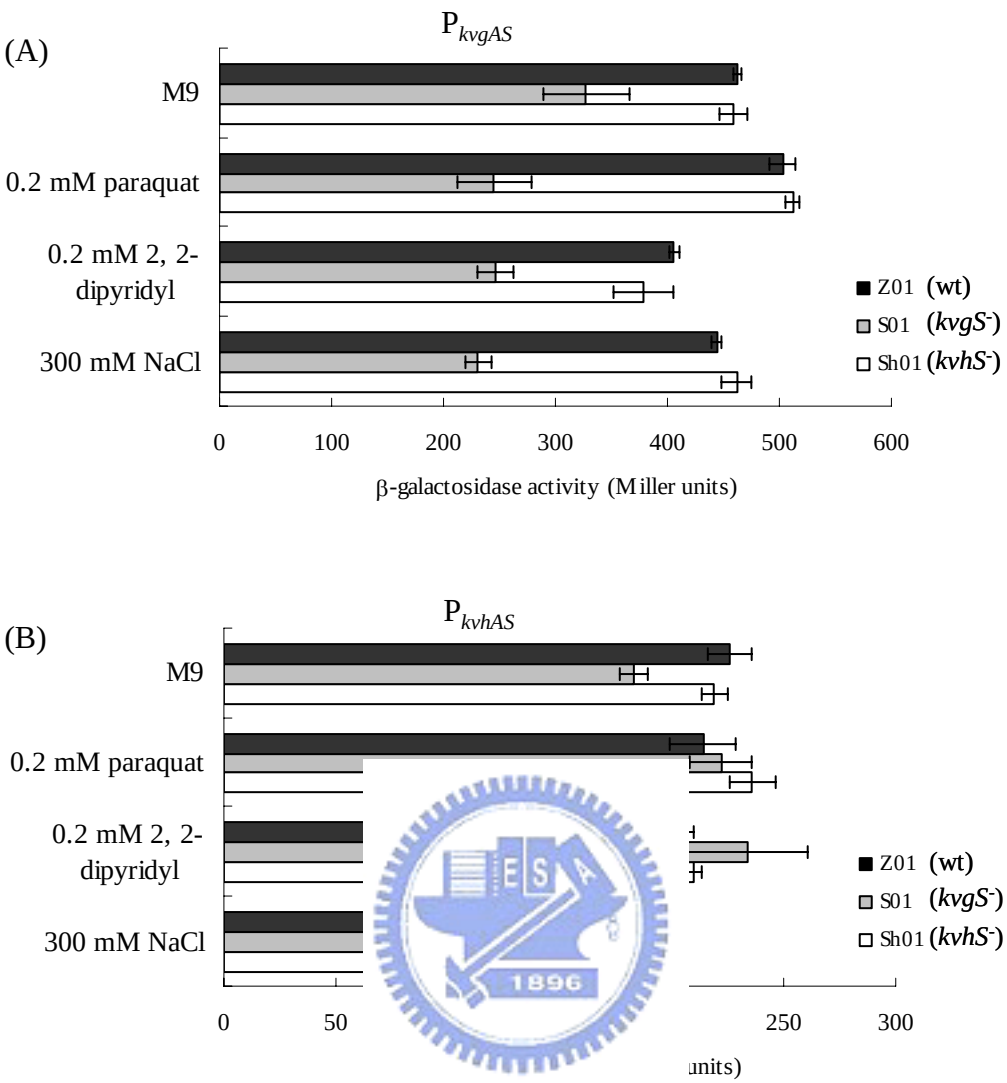
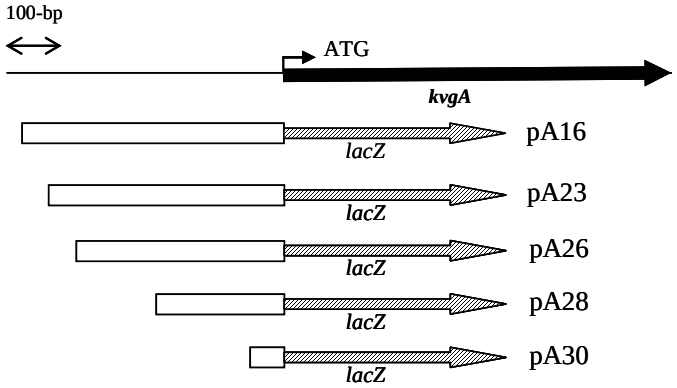
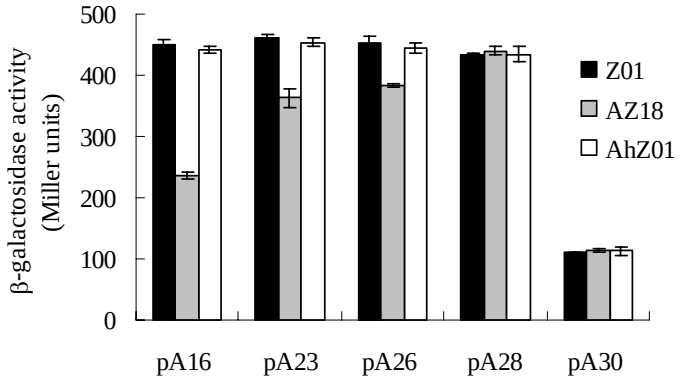


Fig. 5. Effects of different environmental stimuli on *kvgAS* and *kvhAS* promoter activity. Activity of the promoters P_{kvgAS} **(A)** and P_{kvhAS} **(B)** were examined in wild-type (Z01), *kvgS*$^-$ mutant (S01), and *kvhS*$^-$ mutant (Sh01). Bacteria were inoculated in M9 medium or the medium supplemented with different reagents at 37°C for 1 h. The β-galactosidase activity was determined.


100-bp
ATG
kvgA
pA16
lacZ
pA23
lacZ
pA26
lacZ
pA28
lacZ
pA30
lacZ

(A)


β-galactosidase activity
(Miller units)
500
400
300
200
100
0
Z01
AZ18
AhZ01
pA16
pA23
pA26
pA28
pA30

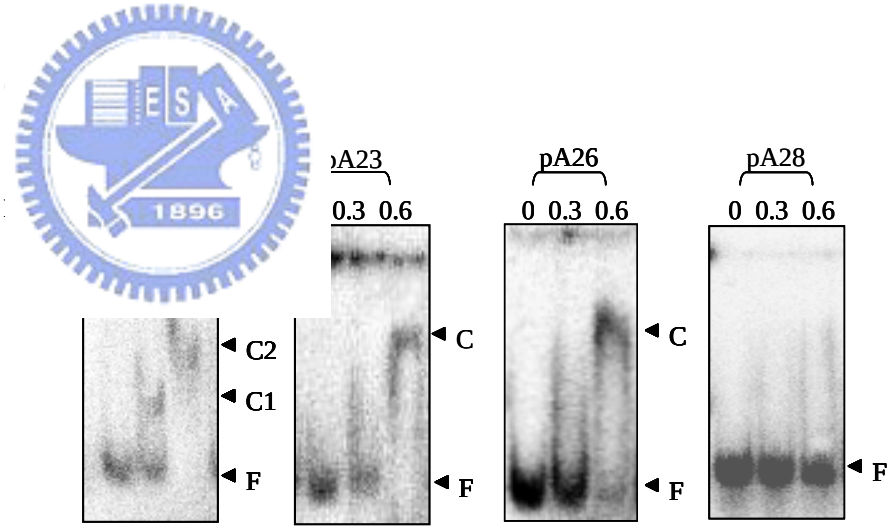
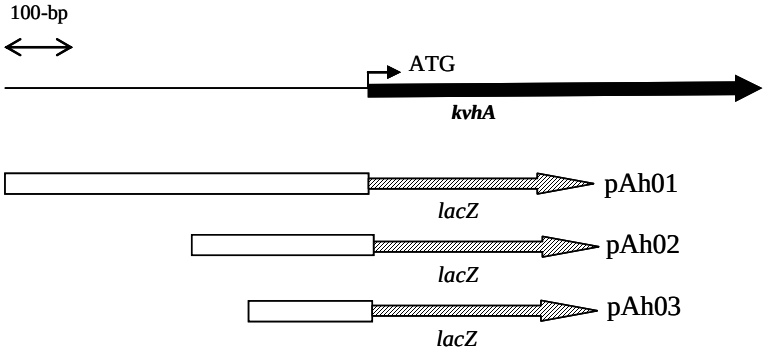
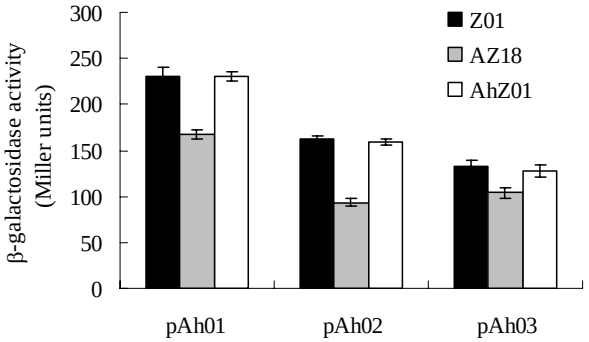

pA23
pA26
pA28
0 0.3 0.6
0 0.3 0.6
0 0.3 0.6
C2
C1
C
C
F
F
F
F

Fig. 6. Identification of KvgA binding region on P_{kvgAS}. ity of *kvgAS* was determined by β-galactosidase activity measurement. The plasmids carrying respectively the P_{kvgA} , pA23, pA26, pA28 and pA30, were transferred into Z01 (wt), AZ18 (*kvgA*$^-$), and AhZ01 (*kvhA*$^-$), respectively by were grown in M9 medium to an OD600 of 0.7, and the β-galactosidase activity was determined. **(B)** EMSA of the P_{kvgAS}. DNA fragments including pA16, pA23, pA26, and pA28 were used as the binding probes. The amounts of protein used are indicated on each lane. The DNA and protein complexes formed are indcated as C and the free probes are indicated as F.


100-bp
ATG
kvhA
pAh01
lacZ
pAh02
lacZ
pAh03
lacZ

(A)


β-galactosidase activity (Miller units)
300
250
200
150
100
50
0
Z01
AZ18
AhZ01
pAh01
pAh02
pAh03

(B)

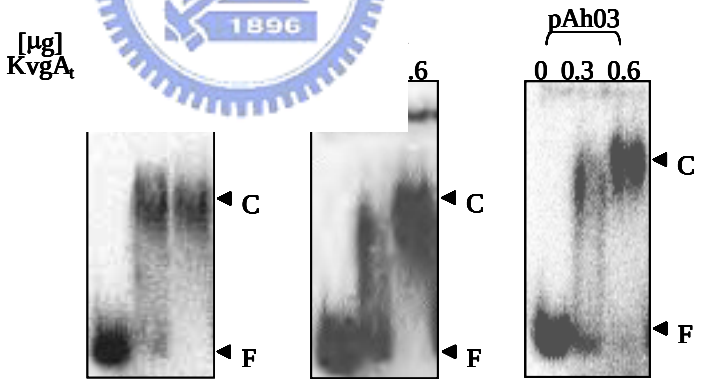

[μg]
KvgA$_t$
pAh03
0 0.3 0.6
C
F
C
F
C
F

Fig. 7. Identification of KvgA binding region on P_{kvhAS}. **(A** ɔf *kvhAS* was determined by β-galactosidase activity assay. The plasmids carrying P_{kvhAS} promoter regions, pAh01, pAh02 and pAh03, were transferred into Z01 (wt), AZ18 (*kvgA*-), and AhZ01 (*kvhA*-), respectively by conjugation. The cells were grown in M9 medium to an OD600 of 0.7, and the β-galactosidase activity was determined. **(B)** EMSA of the KvgAt binding activity to the promoter P_{kvhAS}. DNA fragments including pAh01, pAh02, and pAh03 were used as the binding probes. The amounts of protein used are indicated on each lane. The DNA and protein complexes formed are indicated as C and the free probes are indicated as F.

Fig. 8. Sequence analysis of *P* The transcription initiation site (+1) identified is indicated by and -35 sequences are boxed. The translation start codon (RpoS binding element are in boldface and underlined. The shaded sequence contains the regulatory region of KvgA. Each of the promoter constructs is labeled and indicated by vertical arrow. The horizontal arrows indicate the positions and directions of the gene specific primers that were used for PCR amplification in 5'-RACE.

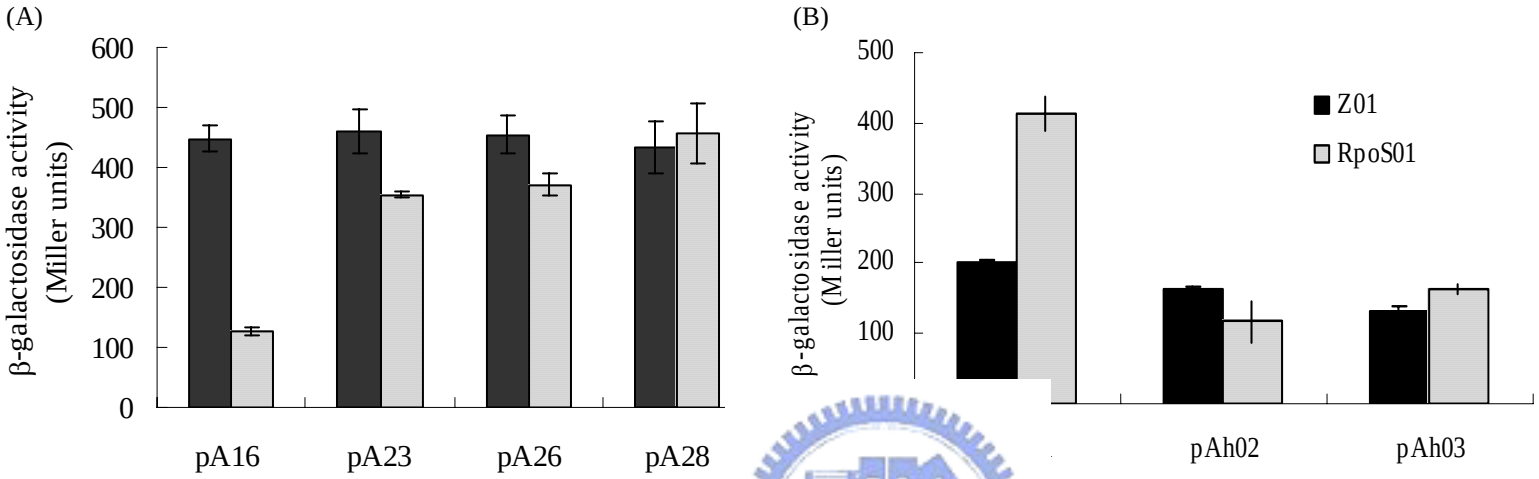


Fig. 9. Deletion of *rpoS* affects the expression of *kvgAS* and *kvhAS* promoters. The promoters of *kvgAS* **(A)** and *kvhAS* **(B)** were determined by β-galactosidase activity assay. The plasmids carrying P_{kvgA} including pA16, pA23, pA26 and pA28, and P_{kvhAS} including pAh01, pAh02 and pAh03, were transferred into Z01 and RpoS01 respectively by conjugation. The cells were grown in M9 medium to an OD600 of 0.7, and the β-galactosidase activity was determined.

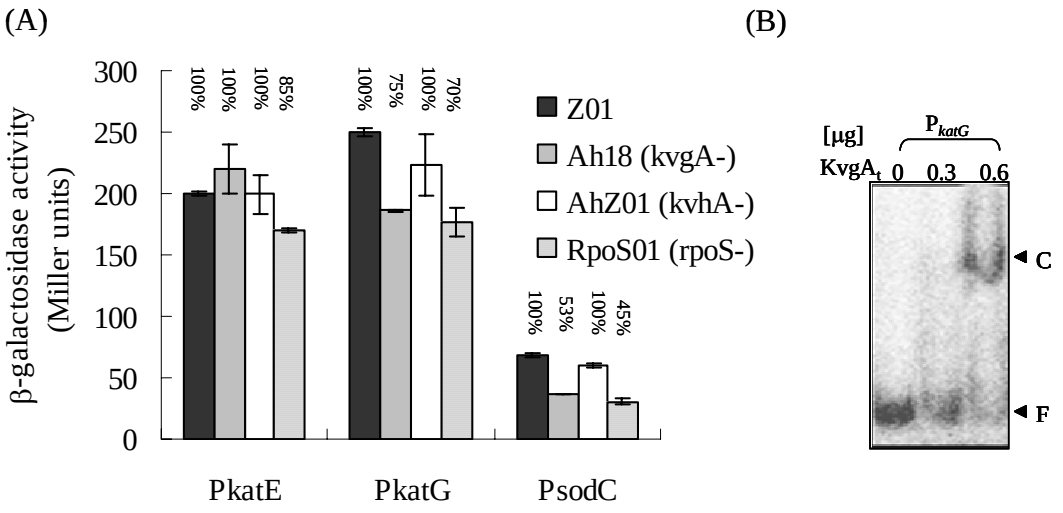


Fig. 10. (A) Deletion effects of *kvgA*, *kvhA* and *rpoS* on expression *katG*, *katE*, and *sodC*. The promoter activities of the stress related genes, *katG*, *katE*, and *sodC*, were determined by β-galactosidase activity assay. The plasmids carrying each of the promoters pKatE, pKatG, and into Z01 (wt), AZ18 (*kvgA*$^-$), and AhZ01 (*kvhA*$^-$), and RpoS y conjugation. The bacteria of stationary phase cultures were ıctosidase activity determined. **(B)** EMSA of the $KvgA_t$ bind ;ment of the P_{katG} was labeled with [γ -^{32}P]ATP and the recombinant $KvgA_t$, were added to the binding assay mixture. The amounts of protein used are indicated on each lane. Specific competition was performed by adding the unlabelled DNA fragments into the mixture (lane 4). The unlabelled pUC19 DNA was also added as a non-specific competitor to the sample in lane 5. The DNA and protein complexes formed are indicated as C and the free probes are indicated as F.

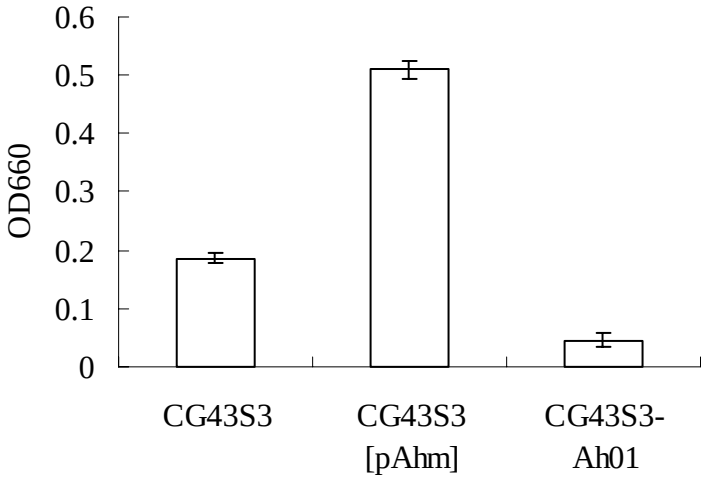
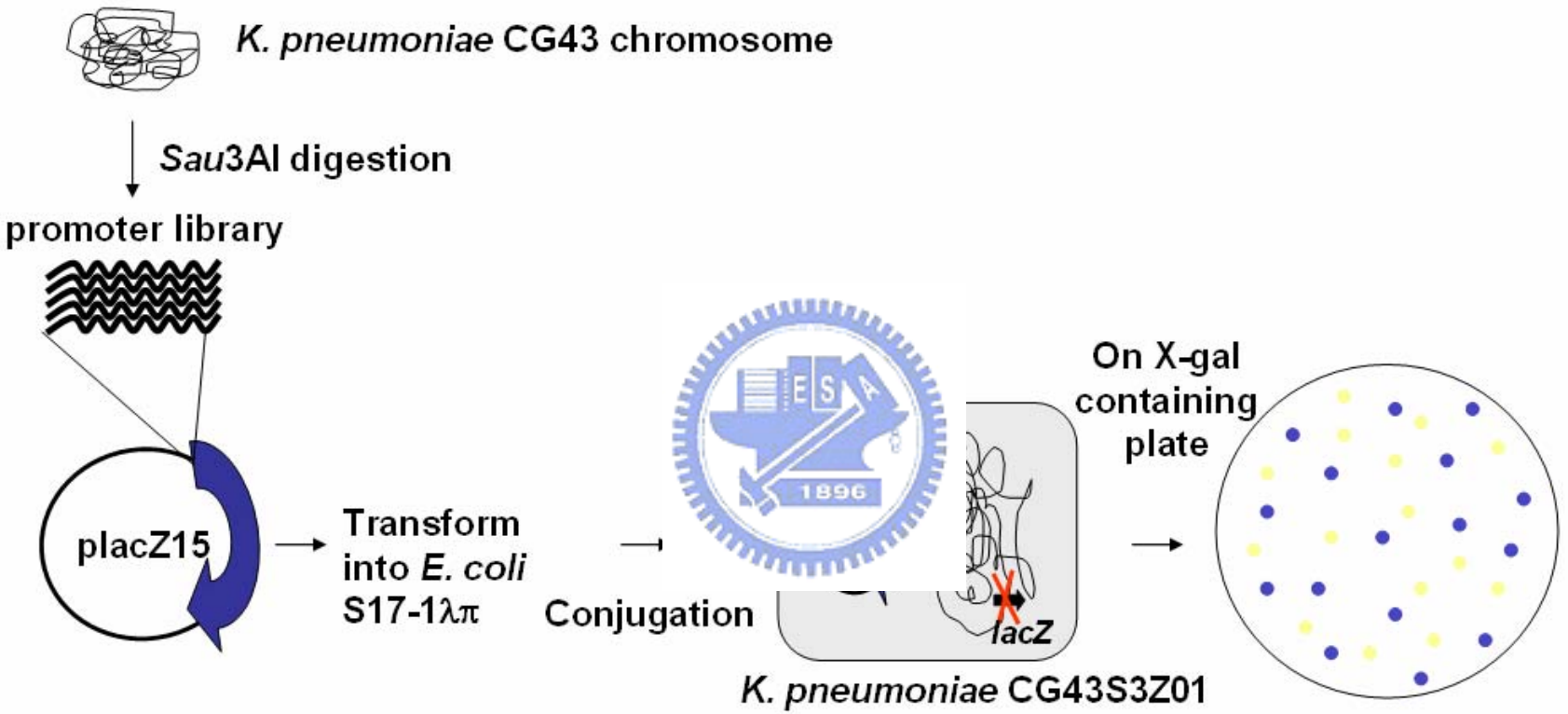


Fig. 11. Effect of MurA activity on *K. pneumoniae* CG43S3, CG43S3[pAhm], and CG43S3-Ah01. The MurA activities from whole cell lysates of bacteria were detected the release of inorganic phosphate by adding the color reagent and the absorbance at OD_{660} (described in Materia[illegible]ganic phosphate release was measured in triplicate.


K. pneumoniae CG43 chromosome
Sau3AI digestion
promoter library
placZ15
Transform into E. coli S17-1λπ
Conjugation
lacZ
K. pneumoniae CG43S3Z01
On X-gal containing plate

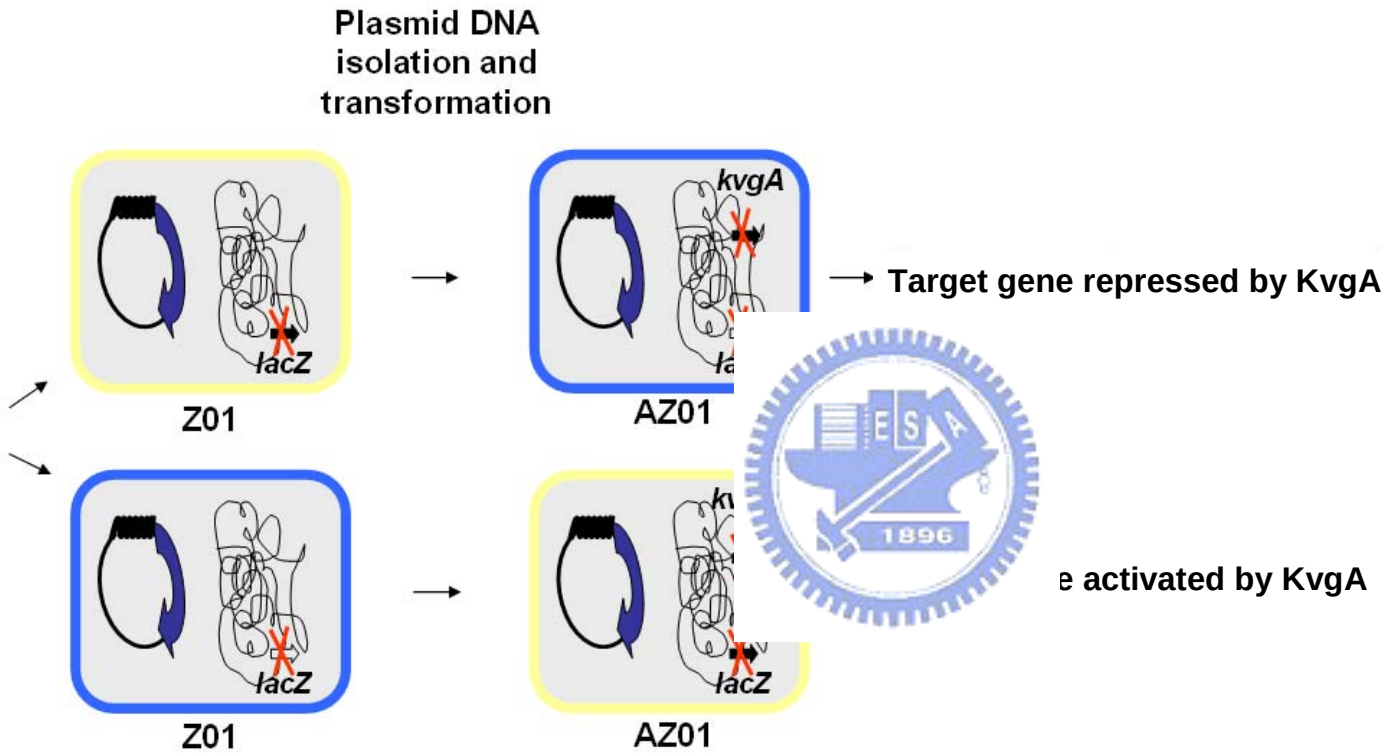


Fig. 12. The strategy to identify the KvgA-regulated genes

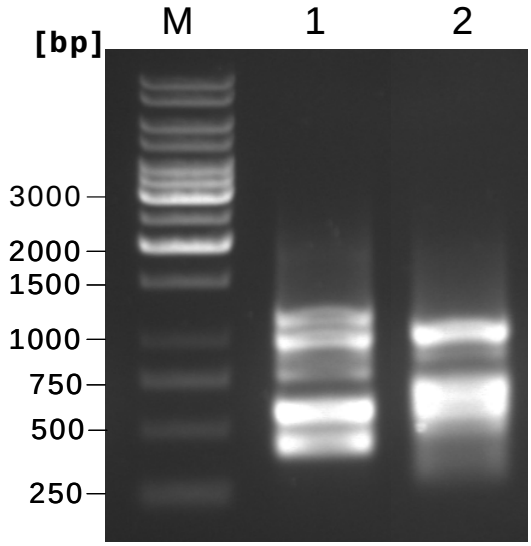
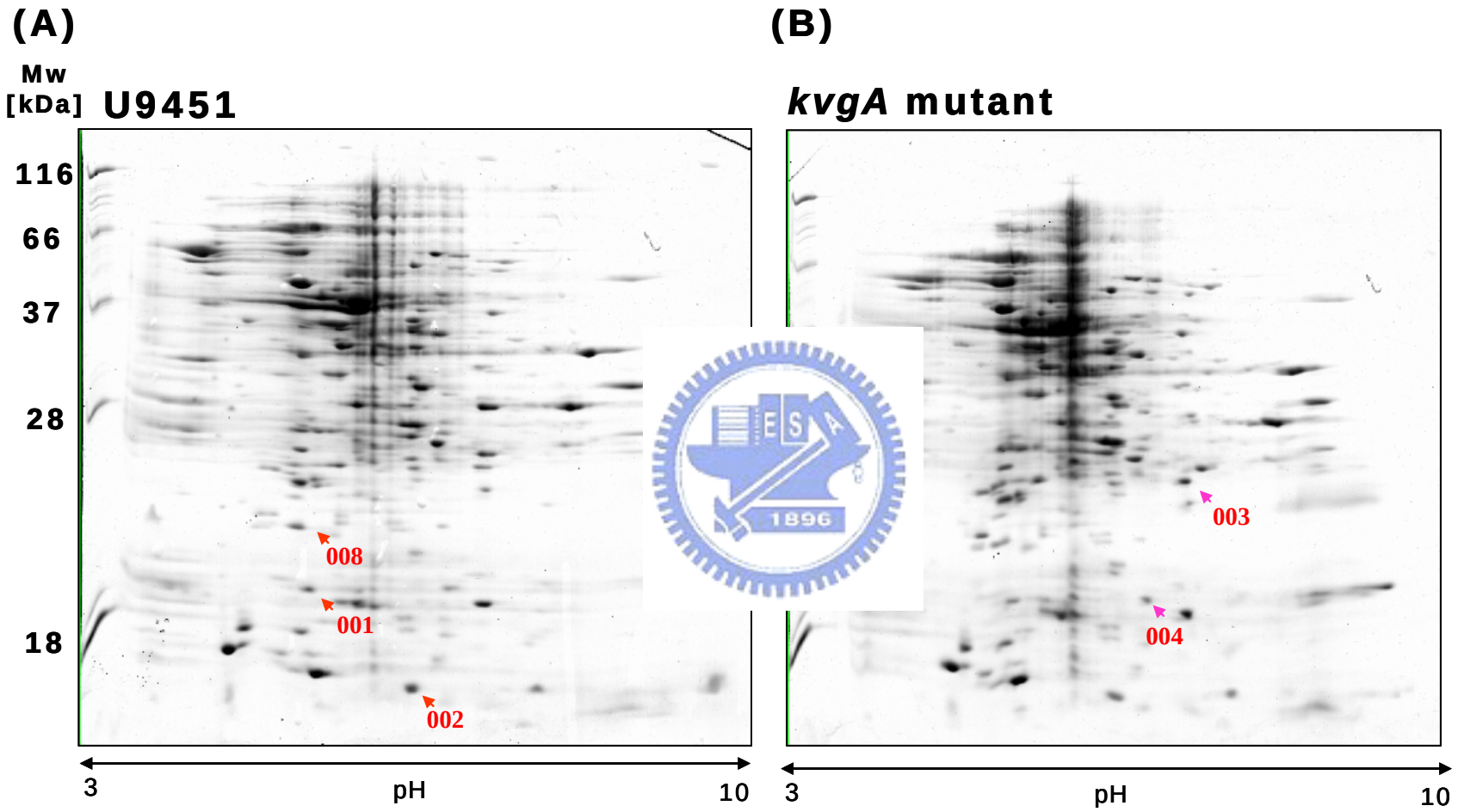
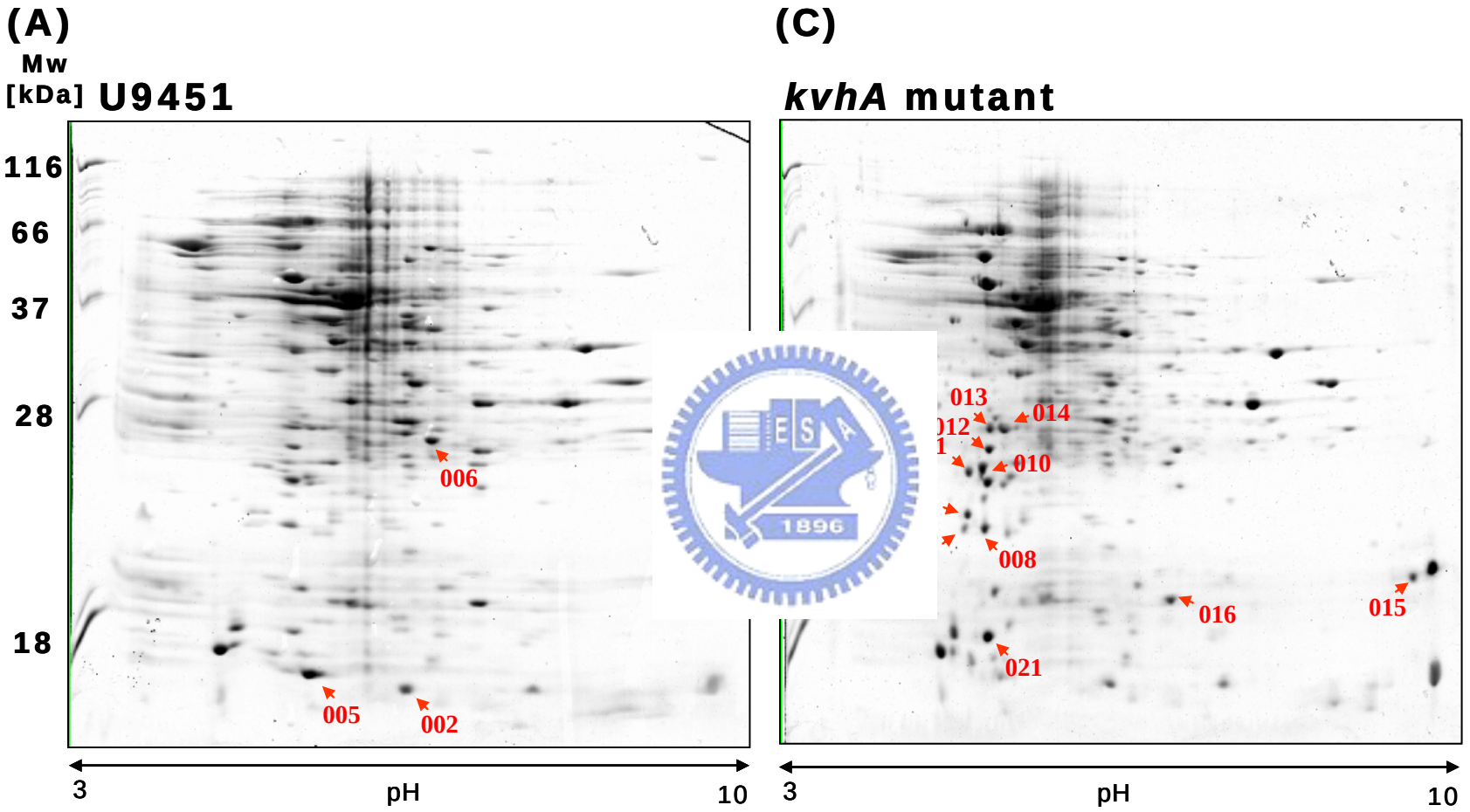


Fig. 13. The PCR-amplified DNA patterns of cDNA substractive hybridization analysis of wild type strain versus *kvhA* deletion mutant. The bacteria were grown in M9 medium. After the cDNA [illegible] on analysis, the differentially expressed gene products were [illegible] agarose gel and stained with ethidium bromide. Lane 1: Kv [illegible] licon. Lane 2: KvhA activated DNA amplicon.


(A)
Mw
[kDa]
U9451
116
66
37
28
18
008
001
002
3
pH
10
(B)
kvgA mutant
003
004
3
pH
10


(A)
Mw
[kDa]
U9451
116
66
37
28
18
006
005
002
3
pH
10
(C)
kvhA mutant
013
014
012
010
008
016
015
021
3
pH
10

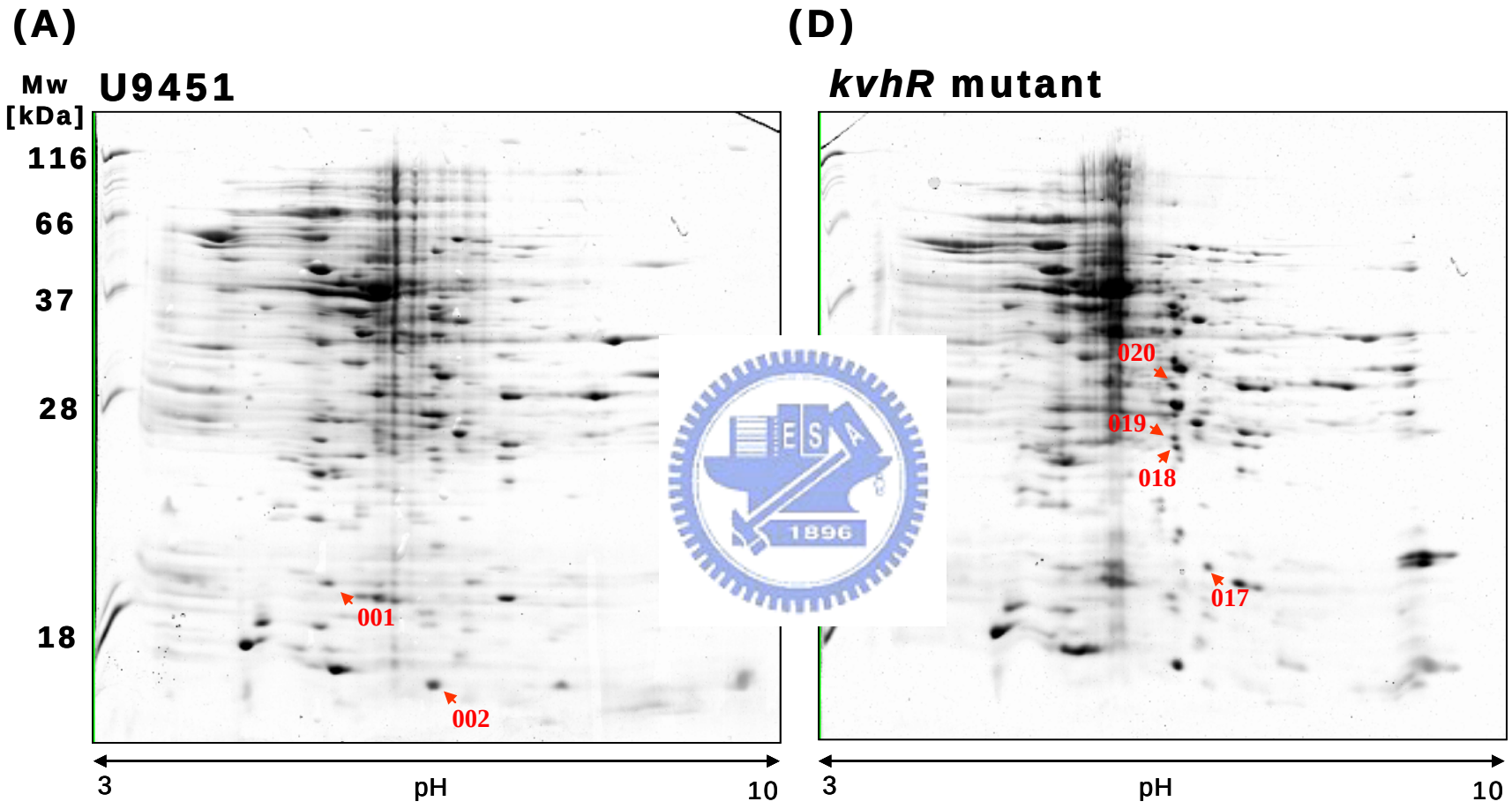

(A)
U9451
Mw
[kDa]
116
66
37
28
18
001
002
3
pH
10
(D)
kvhR mutant
020
019
018
017
3
pH
10

Fig. 14. 2D protein gel electrophoresis patterns (pH gradient 3 to 10) of *K. pneumoniae* U9451 **(A)** and its isogenic mutants, *kvgA* **(B)**, *kvhA* **(C)** and *kvhR* **(D)**. Five hundred micrograms of protein extracts from late-log phase culture in M9 medium were analyzed and stained with Coomassie blue. The arrowheads indicate spots with differential expression in wild and mutants.The numbers of spots were analyzed the protein identity through MALDI/TOF mass spectroscopy and the results shown in Table 8.

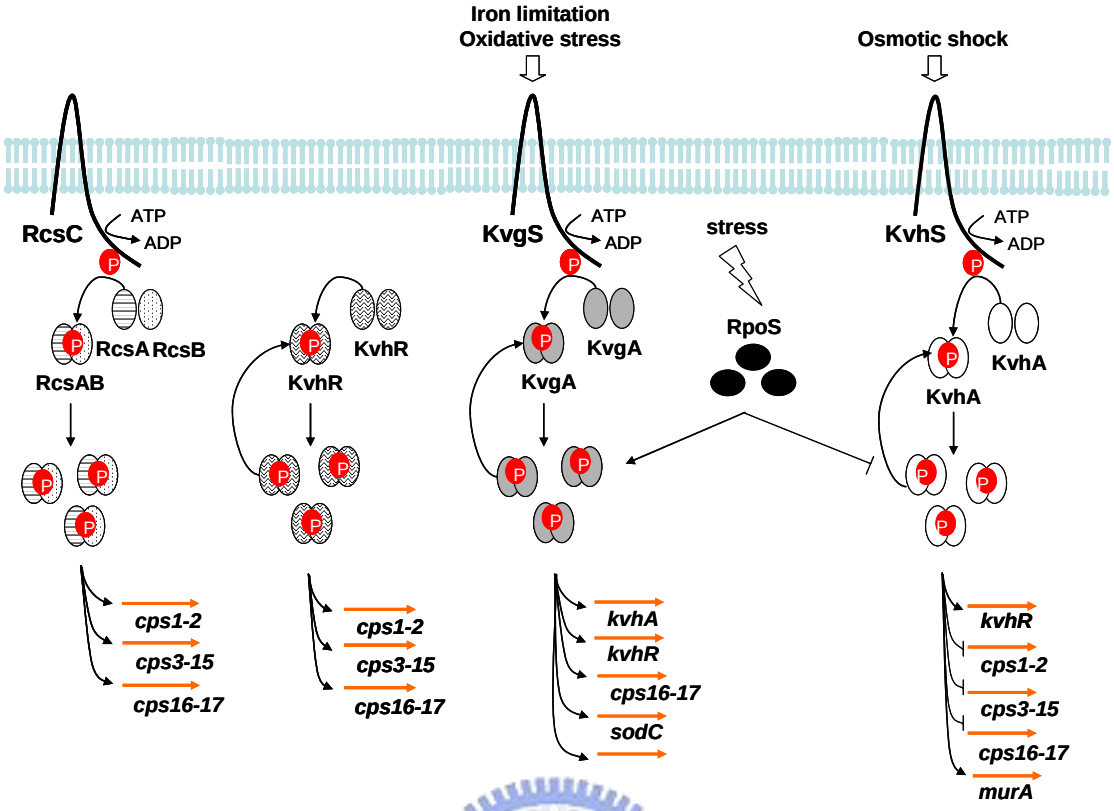


Fig. 15. A model of KvgAS, K ory circuits.