SUPPLEMENTAL MATERIAL

Supplemental Figures

Figure S1. The H-NS regulator encoded in pAB5 is 100% identical to the H-NS encoded in other LCPs. Amino acids alignment of the H-NS regulators encoded in the LCPs pAB5, pAB3 and pAB4 and the H-NS encoded in UPAB1 chromosome.

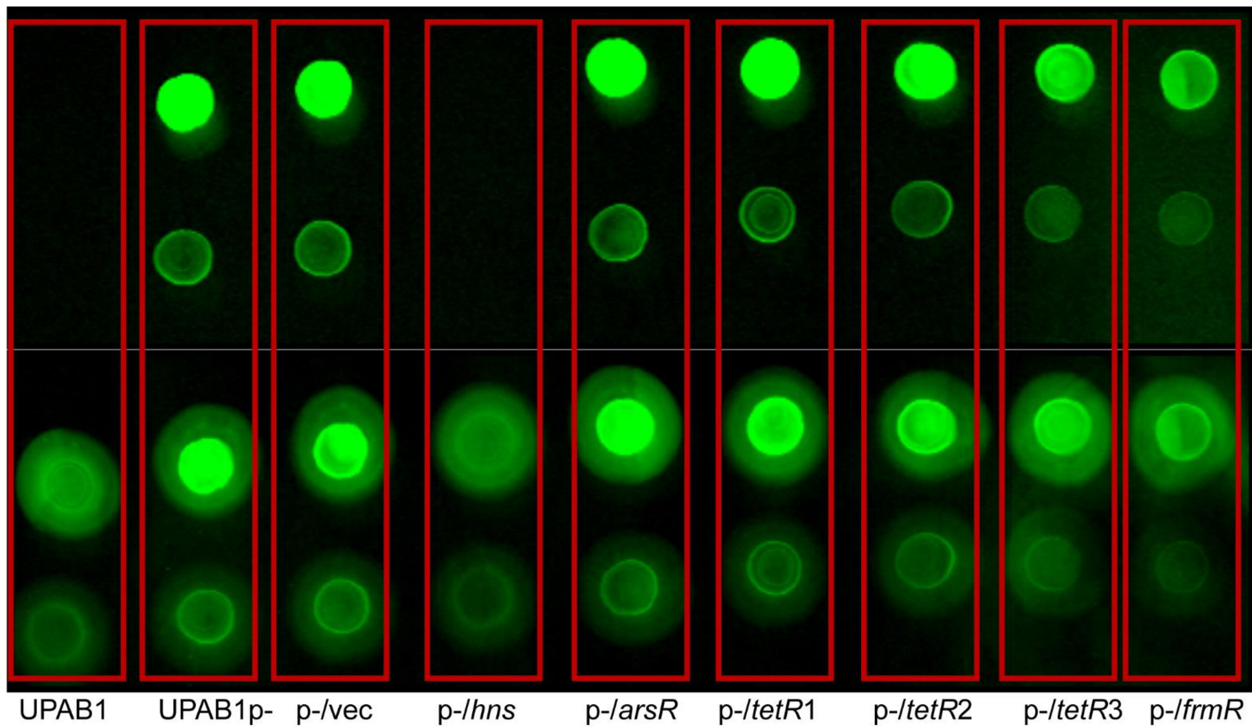


Figure S2. H-NS reduce PNAG production. UPAB1, UPAB1p-, and UPAB1p- empty vector (p-/vec) or the vector expressing the pAB5 regulators. Immunoblots using antibodies anti-PNAG (A) and antibodies anti-UPAB1 as loading control (B). Cells were taken from overnight LB-agar plates incubated at 26 °C and adjusted to OD 1.

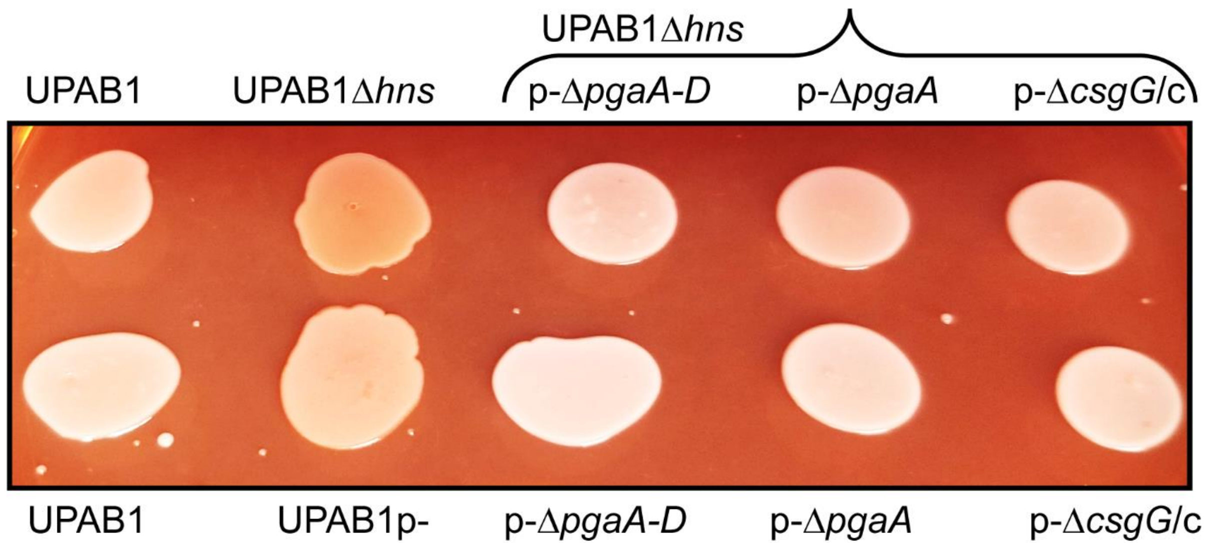
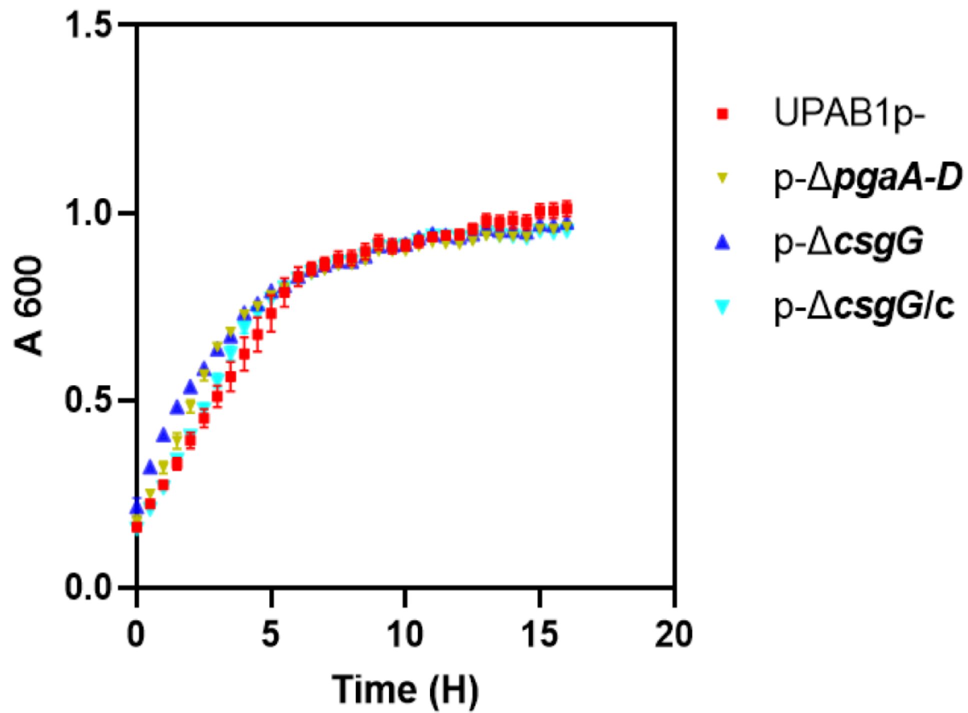


Figure S3. **PgaA-D and CsgG like clusters are involved in Congo-red binding.** Deletions of *pgaA-D*, *pgaA* and *csgG* were made in UPAB1Δ*hns* background (top panel) and UPAB1p- background. Cells were incubated for 48 hours at 26°C on YESCA-Congo-red agar plates.



FigureS4.

Growth curves of UPAB1p- and derivative mutant strains in YESCA-DMSO media, measured by OD600. The graphs represent the mean and standard deviation of three replicates.

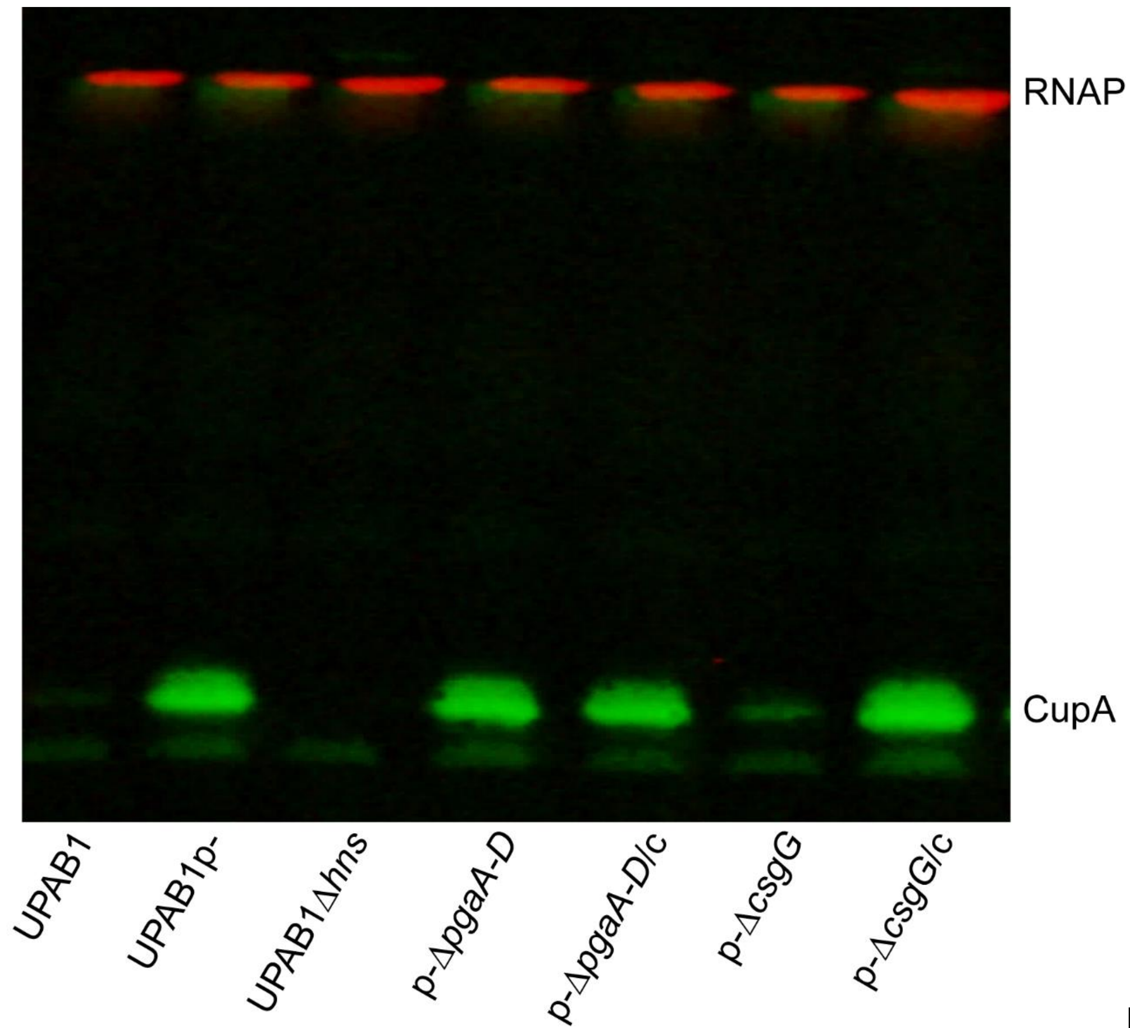


Figure S5. pAB5 inhibits CUP pili formation. Western blot of OD-normalized whole cell. Western blot probing for CupA (the Usher protein from Cup 2 pili). RNAP is included as loading control.

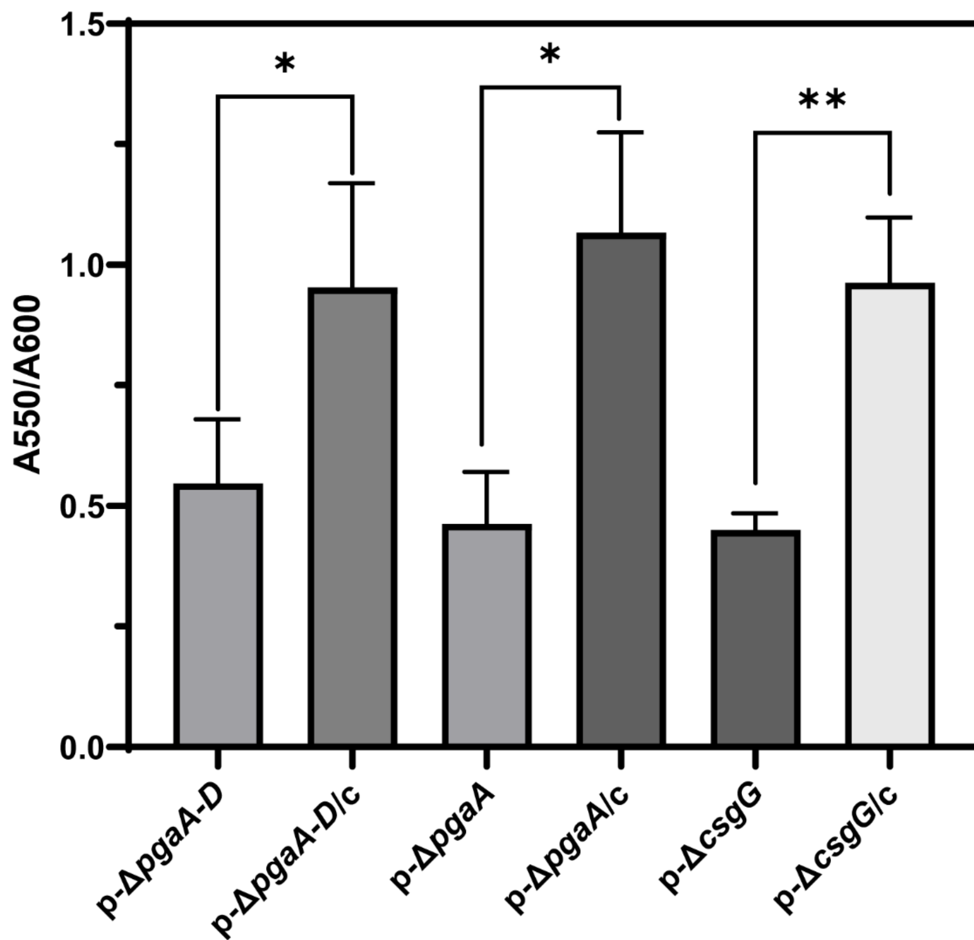


Figure S6. **Biofilm formation in UPAB1p- mutants and complemented strains.** Cells were grown for 8 hours on LB broth at 37°C under static conditions. Biofilm formation was measured by the crystal violet binding and normalized to the OD600. The values represent the mean and standard deviations from three independent experiments. Statistical analysis by t test was performed by comparison with the pAB5- strain (** $p \leq 0.005$, * $p \leq 0.05$).

Table S1. List of regulators identified in pAB5

Accession number	Description	Protein name
D1G37_RS18580	H-NS histone family protein	H-NS
D1G37_RS18620	TetR/AcrR family transcriptional regulator	TetR1
D1G37_RS18650	TetR/AcrR family transcriptional regulator	TetR2
D1G37_RS18810	TetR family transcriptional regulator	TetR3
D1G37_RS18815	ArsR family transcriptional regulator	ArsR
D1G37_RS18965	metal/formaldehyde-sensitive family transcriptional regulator	FrmR

Table S2. Bacterial strains used in this study.

Strain	Relevant properties	Reference
UPAB1	MDR Urine isolate with pAB5 plasmid	(1)
UPAB1p- (p-)	UPAB1derivative strain without pAB5	(1)
UPAB1Δ*hns*	UPAB1 containing an unmarked *hns* deletion	this study
p-/vec	UPAB1p- containing the pVRL2 expression vector	this study
p-/*hns*	p- expressing *hns* in pVRL2	this study
p-/*tetR*1	p- expressing *tetR*1 in pVRL2	this study
p-/*tetR*2	p- expressing *tetR*2 in pVRL2	this study
p-/*tetR*3	p- expressing *tetR*3 in pVRL2	this study
p-/*ars*R	p- expressing *arsR* in pVRL2	this study
p-/*frm*R	p- expressing *frm*R in pVRL2	this study
p-Δ*pgaA-D*	p- containing an unmarked *pgaA-D* deletion	this study
p-Δ*pgaA*	p- containing an unmarked *pgaA* deletion	this study
p-Δ*csgG*	p- containing an unmarked *csgG* deletion	this study
p-ΔAb*12600*	p- containing an unmarked D1G37_12600 deletion	this study
p+Δ*hns* Δ*pgaA-D*	UPAB1 containing an unmarked *hns* and *pgaA-D* deletion	this study
p+Δ*hns* Δ*pgaA*	UPAB1 containing an unmarked *hns* and *pgaA* deletion	this study
p+Δ*hns* Δ*csgG*	UPAB1 containing an unmarked *hns* and *csgG* deletion	this study
p-Δ*pgaA-D*/c	p-Δ*pgaA-D* complemented	this study
p-Δ*pgaA*/c	p-Δ*pgaA* complemented	this study
p-Δ*csgG*/c	p-ΔcsgG complemented	this study

Table S3. Bacterial plasmids used in this study.

Plasmid	Relevant properties	Reference
pVRL2	Expression vector in Acinetobacter, Gm^r	(2)
pVRL2/*hns*	pVRL2 containing *hns*	this study
pVRL2/*tetR1*	pVRL2 containing *tetR1*	this study
pVRL2/*tetR2*	pVRL2 containing *tetR2*	this study
pVRL2/*tetR3*	pVRL2 containing *tetR3*	this study
pVRL2/*arsR*	pVRL2 containing *arsR*	this study
pVRL2/*frmR*	pVRL2 containing *frmR*	this study
pAT03H	pMMB67EH with FLP recombinase, Hyg^r	Feldman lab
pAT04H	pMMB67EH with RecAb recombinase, Hyg^r	Feldman lab
pUCT18T-miniTn7-Gm	mobilizable mini-Tn7 vector, Gm^r	(3)
pUCT18T-miniTn7-*pgaA-D*	for Δ*pgaA-D* complementation	this study
pUCT18T-miniTn7-*pgaA*	for Δ*pgaA* complementation	this study
pUCT18T-miniTn7-*csgG*	for Δ*csgG* complementation	this study
pTNS2	Tn7 transposase-expressing helper plasmid, Amp^r	(4)
pRK2013	RK2 derivative, Kmr, self-transmissible	(5)
pET28 a+	Kmr	Novagen
pET28 a-CupA	pET28a+ expressing *cupA* from UPAB1	this study

Table S4. Oligonucleotides used in this study.

Name	Sequence[a]	gene target
Cloning in pVRL2 (gene expression)		
HNS xhoI Fw	CCG**CTCGAG**ATGATGAAAGAATTTAATTTAG	*hns*
HNS pstI Rv	AAAA**CTGCAG**TTATTTAATCAGGAATTCACTCA	
*tetR*1 xhoI Fw	CCG**CTCGAG**ATGACAAATAAAGCTTCACAGC	te*tR*1
*tetR*1 pstI Rv	AAAA**CTGCAG**TCAAAATTGCTCACCATATAAA	
*tetR*2 xhoI Fw	CCG**CTCGAG**ATGACTAAAGTTATTTCAAAAA	*tetR*2
*tetR*2 pstI Rv	AAAA**CTGCAG**TTAAGCCTCAAATGTCTGAATTA	
*tetR*3 xhoI Fw	CCG**CTCGAG**ATGATGTCTAGATTAGATAAAAGTA	*tetR*3
*tetR*3 pstI Rv	AAAA**CTGCAG**TTAAGACCCACTTTCACATTT	
arsR xhoI Fw	CCG**CTCGAG**ATGGGGATCTCCTTAAACCATG	*arsR*
arsR pstI Rv	AAAA**CTGCAG**TTAAATGTGAAAGTGGGTCTTA	
frmR xhoI Fw	CCG**CTCGAG**ATGCCTAATCAGGTTGAAGACAA	*frmR*
frmR pstI Rv	AAAA**CTGCAG**CTATTTTAAATATGATTTTAGA	
Mutant construction		
Hns2 FP1	AATTCGAGCTCGGTACCCGGGGATCAACA GATGATTCTATATATACGATT	*hns* deletion
Hns2 RP1	AGCAGCTCCAGCCTACACAATCGCTTAAT ACTCTATATGTTTTAA	*hns* deletion
Hns2 FP3	AGGAACTAAGGAGGATATTCATATGTGAT GTATAAAAAAAGCCTA	*hns* deletion
Hns2 RP3	GCCTGCAGGTCGACTCTAGAGGATCCG GCAAGCGCATGCGGTGATT	*hns* deletion
pgaA Up Fw	AATTCGAGCTCGGTACCCGGGGATCGCT AATTGTTTTAAGTCCATA	*pgaA* deletion

[a]Engineered resctriction enzyme sites are indicated in bold.

Table S4. Oligonucleotides used in this study (continued).

Name	Sequence[a]	gene target
Mutant construction		
pgaA Up Rv deletion	AGCAGCTCCAGCCTACACAATCGCTGGC AGCTATCACGGACATGGC	*pgaA*
pgaA Dw Fw deletion	TAGGAACTAAGGAGGATATTCATATGAGTT TGTCCTGCTCTATAAT	*pgaA*
pgaA Dw Rv deletion	GCCTGCAGGTCGACTCTAGAGGATCTAAT CTGGATGTGTTTGAAGG	*pgaA*
pnag Up Fw deletion	AATTCGAGCTCGGTACCCGGGGATCGAAC ACGGCAGAACAACTTTCT	*pgaA-D*
pnag Up Rv deletion	AGCAGCTCCAGCCTACACAATCGCTTATGG TCAGTACCGAATCATT	*pgaA-D*
pnag Dw Fw deletion	TAGGAACTAAGGAGGATATTCATATTATAAT TTTGAATAGCTTGCT	*pgaA-D*
pnag Dw Rv deletion	GCCTGCAGGTCGACTCTAGAGGATCTAATC TGGATGTGTTTGAAGG	*pgaA-D*
12595 FP1 deletion	GAATTCGAGCTCGGTACCCGGGGATCGGT AGCCTCTACCTATTGGAT	*csgG*
12595 RP1 deletion	AGCAGCTCCAGCCTACACAATCGCTGTTTA CTCTATAAAGATAAATAAAT	*csgG*
12595 FP3 deletion	AATAGGAACTAAGGAGGATATTCATATGTTT TGGATATGTGTTAGATGA	*csgG*
12595 RP3 deletion	GCCTGCAGGTCGACTCTAGAGGATCAACAA GTT TTTTCTCACCTGTCC	*csgG*
12600 FP1 deletion	GAATTCGAGCTCGGTACCCGGGGATCCATAT	Ab12600

	AATTAACTATCTATAGAATAA	
12600 RP1 deletion	AGCAGCTCCAGCCTACACAATCGCTCTAACA CATATCCAAAATTATTGAA	Ab12600
12600 FP3	AATAGGAACTAAGGAGGATATTCATATGATG ATTAAGAAACTTCTCATT	Ab12600 deletion
12600 RP3	GCCTGCAGGTCGACTCTAGAGGATCGTAA ATACCGTTTTAAACGGAAG	Ab12600 deletion

[a]Engineered resctriction enzyme sites are indicated in bold.

Table S4. Oligonucleotides used in this study (continued).

Name	Sequence[a]	gene target
<u>pUC18TminiTn7T-Gm (strain complementation)</u>		
pgaA Fw hind3	CTGCAGGAATTCCTCGAG**AAGCTT**GGGT TAAGTCAATTATTTATTTTAACC	*pgaA*/*pgaA-D* complementation
pgaA Rv kpni	GCAAGGCCTTCGCGA**GGTACC**GGGTTT T TGGTAAATCTTTTTCTA	*pgaA* complementation
pnag Rv kpni	GCAAGGCCTTCGCGA**GGTACC**GGGTCA TTTCAAATCATATCGAGT	*pgaA-D* complementation
12595 Fw hind3	CTGCAGGAATTCCTCGAG**AAGCTT**GGGA AGTTGAGCTATTGTCCTCTG	*csgG* complementation
12595 Rv kpni	CCTGCAAGGCCTTCGCGA**GGTACC**GGG TTTGATGAGTATAAGTTCCCC	*csgG* complementation
Tn7R	CACAGCATAACTGGACTGATTTC	miniTn7T
GlmS1 UPAB1	TTTGCTGATGAAAATAGCGG	insertion
<u>RT-PCR</u>		
RT *rpoB* F	CTAACGGTGTGCCGGTAGAT	*rpoB*
RT *rpoB* R	TCTTGCTCACCGCCTACTTT	
RT 510 F	GCTTGTCCACCATCCGTACT	*pgaA*
RT 510 R	AAGACCGTGAAATGGAAACG	
RT 505 F	CCTTGTTTACGCGCTTCTTC	*pgaB*
RT 505 R	GCTGTGCCTTGATCTCAACA	
RT 500 F	AAAAGGCTGCATAAGCCAAA	*pgaC*
RT 500 R	ACAGCACTGCCGAAATTCTT	
RT 12595 F	GGGCAGGCGAAAACTATTCT	*csgG*
RT 12595 R	CCGCGTCCTAAAATACCAAA	

RT 12600 F	TTCTTGCGATGAGTTTGGTG	Ab12600
RT 12600 R	AGCTAGGCCCTTACCTTTGG	

[a]Engineered resctriction enzyme sites are indicated in bold.

Bibliography

1. Di Venanzio G, Flores-Mireles AL, Calix JJ, Haurat MF, Scott NE, Palmer LD, Potter RF, Hibbing ME, Friedman L, Wang B, Dantas G, Skaar EP, Hultgren SJ, Feldman MF. 2019. Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic Acinetobacter baumannii chromosomal genes. Nat Commun 10:1100.

2. Lucidi M, Visaggio D, Prencipe E, Imperi F, Rampioni G, Cincotti G, Leoni L, Visca P. 2019. New shuttle vectors for real-time gene expression analysis in multidrug-resistant Acinetobacter species: In vitro and in vivo responses to environmental stressors. Appl Environ Microbiol 85.

3. Kumar A, Dalton C, Cortez-Cordova J, Schweizer HP. 2010. Mini-Tn7 vectors as genetic tools for single copy gene cloning in Acinetobacter baumannii. J Microbiol Methods 82:296–300.

4. Choi KH, Gaynor JB, White KG, Lopez C, Bosio CM, Karkhoff-Schweizer RR, Schweizer HP. 2005. A Tn7-based broad-range bacterial cloning and expression system. Nat Methods 2:443–448.

5. Figurski DH, Helinski DR. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc Natl Acad Sci U S A 76:1648–52.