TABLE S2. Bacterial strains and plasmids

Strain / plasmid	Genotype or description	Source or reference
Strains		
S. pneumoniae		
Sp1 (Rx1)	unencapsulated wild type, *str1*; *hexA*	(1)
Sp4 (D39)	encapsulated wild type, serotype 2	(2)
Sp57	Rx1, Δ*locZ::lox72*	This work
Sp58	Rx1, *cm*, Δ*stkP::cm*, *bgaA*::P_{czcD}-*gfp-locZ*	This work
Sp60	Rx1, Δ*locZ*::*lox72, bgaA*:: P_{czcD}-*his-locZ*	This work
Sp208 (R6)	unencapsulated wild type	(3)
Sp225	R6, *str*, *rpsL*	This work
Sp227	R6, *str, locZ::janus cassette*	This work
Sp228	R6, *str*, *rfp-locZ*	This work
Sp229	R6, *str*, *gfp-locZ*	This work
Sp234	R6, *str, locZ-T67A/T78A*	This work
Sp235	R6, str, *locZ-T67E/T78E*	This work
Sp239	R6, *str*, Δ*locZ*	This work
Sp240	R6, *str*, *tet,* pBCSMH036	This work
Sp242	R6, *str*, *tet*, *rfp-locZ,* pBCSMH036	This work
Sp243	R6, *str*, *tet*, Δ*locZ*, pBCSMH036	This work
Sp246	R6, *str, tet*, *bgaA*:: P_{czcD}-*gfp-stkP*	This work
Sp248	R6, *str*, *tet*, *rfp-locZ, bgaA*:: P_{czcD}-*gfp-stkP*	This work
Sp249	R6, *str*, *tet*, Δ*locZ*, *bgaA*:: P_{czcD}-*gfp-stkP*	This work

Sp250	R6, *str*, *tet*, *bgaA*:: P_{czcD}-*divIVA-gfp*	This work
Sp253	R6, *str*, *tet*, Δ*locZ, bgaA*:: P_{czcD}-*divIVA-gfp*	This work
Sp254	R6, *str*, *tet*, *bgaA*:: P_{czcD}-*gfp-ftsA*	This work
Sp256	R6, *str*, *tet*, *rfp-locZ*, *bgaA*:: P_{czcD}-*gfp-ftsA*	This work
Sp257	R6, *str*, Δ*locZ*, *bgaA*:: P_{czcD}-*gfp-ftsA*	This work
Sp267	D39, Δ*locZ::lox72*	This work
E. coli		
DH5α	*F- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rk-, mk+) phoA supE44 λ- thi-1 gyrA96 relA1*	Invitrogen
BL21 (DE3)Star	F– *omp*T *hsd*SB(rB–, mB–) *gal dcm rne*131 (DE3)	Invitrogen
Plasmids		
pJWV25	*Amp, tet, bgaA*, P_{czcD}-*gfp+*	(4)
pJWV25-GFP-StkP	*Amp, tet, bgaA*, P_{czcD}-*gfp-stkP*	(5)
pJWV25-GFP-FtsA	*Amp, tet, bgaA*, P_{czcD}-*gfp-ftsA*	(5)
pJWV25-DivIVA-GFP	*Amp, tet, bgaA*, P_{czcD}-*divIVA-gfp*	(5)
pZn-GFP-LocZ	*Amp, tet, bgaA*, P_{czcD}-*gfp-locZ*	This study
pZn-His6-LocZ	*Amp, tet, bgaA*, P_{czcD}-*his6-locZ*	This study
pZn-His6-LocZ-T67A/T78A	*Amp, tet, bgaA, P_{czcD}-his6-locZ*-T67A/T78A	This study
pZn-His6-LocZ-T67E/T78E	*Amp, tet, bgaA, P_{czcD}-his6-locZ*-T67E/T78E	This study

pBCSMH036	*Tet,* P_{sigA}- *icfp-ftsZ*	(6)
pDELstkP	*Amp, kan, ΔstkP::cm*	(7)
pETPHos	*Amp,* pET derivative	(8)
pETPHos-LocZ	*Amp,* pETPHos, *his6-locZ*	(9)
pETPHos-LocZ-T67A	*Amp,* pETPHos, *his6-locZ*-T67A	This study
pETPHos-LocZ-T78A	*Amp,* pETPHos, *his6-locZ*-T78A	This study
pETPHosLocZ-T67A/T78A	*Amp,* pETPHos, *his6-locZ*-T67A/T78A	This study
pETPHosLocZ-T67A/T78A/S80A	*Amp,* pETPHos, *his6-locZ*-T67A/T78A/S80A	This study
pCDFDuet	*Sm*	Novagen
pCDFDuet_locZ/stkP	*Sm, his6-locZ, stkP-kinase domain*	This study
Cheshire cassette	*Erm, loxP/ermAM/*P_{fcsK}*/cre/loxP*	(10)
Janus cassette	*Kan, kan-rpsL* $^{+}$	(11)

Amp: ampicillin resistance marker; Cm: chloramphenicol resistance marker; Kan: kanamycin resistance marker; Tet: tetracycline resistance marker, Erm: erythromycin resistance marker; Str: streptomycin resistance marker; Sm: spectinomycin resistance marker

References

1. **Morrison, D. A., M. C. Trombe, M. K. Hayden, G. A. Waszak, and J. D. Chen**. 1984. Isolation of transformation-deficient *Streptococcus pneumoniae* mutants

defective in control of competence, using insertion-duplication mutagenesis with the erythromycin resistance determinant of pAM beta 1. J.Bacteriol. **159**:870-876.

2. **Avery, O. T., C. M. Macleod, and M. McCarty**. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types : induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J.Exp.Med. **79**:137-158.

3. **Hoskins, J., W. E. Alborn, Jr., J. Arnold, L. C. Blaszczak, S. Burgett, B. S. DeHoff, S. T. Estrem, L. Fritz, D. J. Fu, W. Fuller, C. Geringer, R. Gilmour, J. S. Glass, H. Khoja, A. R. Kraft, R. E. Lagace, D. J. LeBlanc, L. N. Lee, E. J. Lefkowitz, J. Lu, P. Matsushima, S. M. McAhren, M. McHenney, K. McLeaster, C. W. Mundy, T. I. Nicas, F. H. Norris, M. O'Gara, R. B. Peery, G. T. Robertson, P. Rockey, P. M. Sun, M. E. Winkler, Y. Yang, M. Young-Bellido, G. Zhao, C. A. Zook, R. H. Baltz, S. R. Jaskunas, P. R. Rosteck, Jr., P. L. Skatrud, and J. I. Glass**. 2001. Genome of the bacterium *Streptococcus pneumoniae* strain R6. J.Bacteriol. **183**:5709-5717.

4. **Eberhardt, A., L. J. Wu, J. Errington, W. Vollmer, and J. W. Veening**. 2009. Cellular localization of choline-utilization proteins in *Streptococcus pneumoniae* using novel fluorescent reporter systems. Mol.Microbiol. **74**:395-408.

5. **Beilharz, K., L. Novakova, D. Fadda, P. Branny, O. Massidda, and J. W. Veening**. 2012. Control of cell division in *Streptococcus pneumoniae* by the conserved Ser/Thr protein kinase StkP. Proc.Natl.Acad.Sci.U.S.A.

6. **Henriques, M. X., M. J. Catalao, J. Figueiredo, J. P. Gomes, and S. R. Filipe**. 2013. Construction of improved tools for protein localization studies in *Streptococcus pneumoniae*. PLoS.One. **8**:e55049.

7. **Novakova, L., L. Saskova, P. Pallova, J. Janecek, J. Novotna, A. Ulrych, J. Echenique, M. C. Trombe, and P. Branny**. 2005. Characterization of a eukaryotic type serine/threonine protein kinase and protein phosphatase of *Streptococcus pneumoniae* and identification of kinase substrates. FEBS J. **272**:1243-1254.

8. **Canova, M. J., L. Kremer, and V. Molle**. 2008. pETPhos: a customized expression vector designed for further characterization of Ser/Thr/Tyr protein kinases and their substrates. Plasmid **60**:149-153.

9. **Novakova, L., S. Bezouskova, P. Pompach, P. Spidlova, L. Saskova, J. Weiser, and P. Branny**. 2010. Identification of multiple substrates of the StkP Ser/Thr protein kinase in *Streptococcus pneumoniae*. J.Bacteriol. **192**:3629-3638.

10. **Weng, L., I. Biswas, and D. A. Morrison**. 2009. A self-deleting *Cre-lox-ermAM* cassette, Cheshire, for marker-less gene deletion in *Streptococcus pneumoniae*. J.Microbiol.Methods **79**:353-357.

11. **Sung, C. K., H. Li, J. P. Claverys, and D. A. Morrison**. 2001. An *rpsL* cassette, janus, for gene replacement through negative selection in *Streptococcus pneumoniae*. Appl.Environ.Microbiol. **67**:5190-5196.