**S1 Table. Bacterial strains and plasmids used in this study.**

|  |  |  |
| --- | --- | --- |
| **Strain** | **Relevant genotype** | **Reference/Source** |
| ***S. pneumoniae*** |  |  |
| D39 | serotype 2 strain, *cps2* | (a) |
| D39∆*galK* | D39 ∆*galK::spe*, SpeR | This work |
| D39∆*lacD* | D39 ∆*lacD::spe,* SpeR | This work |
| D39∆*manA* | D39 ∆*manA::spe,* SpeR | This work |
| D39∆*nagA* | D39 ∆*nagA::spe,* SpeR | This work |
| D39∆*lacDΔgalK* | D39 ∆*lacD::spe* ∆*galK::tmp,* SpeR*,* TmpR | This work |
| D39∆*galT-2* | D39 ∆*galT-2::spe*, SpeR | This work |
| D39∆*galK* + pKB01-*galK* | D39 ∆*galK::spc, bgaA::*P*zn-galK-sfgfp(iGEM)*, SpeR,TetR | This work |
| D39∆*lacD* + pKB01-*lacD* | D39 ∆*lacD::spc, bgaA::*P*zn-lacD-sfgfp(iGEM),* SpeR,TetR | This work |
| D39∆*manA* + pKB01-*manA* | D39 ∆*manA::spc, bgaA::*P*zn-manA-sfgfp(iGEM)*,SpeR,TetR | This work |
| D39∆*nagA* + pKB01-*nagA* | D39∆*nagA::spc, bgaA::*P*ownnagA-nagA-sfgfp(iGEM)*, SpeR,TetR | This work |
| D39∆*galK* + pKB01-*galT-2* | D39∆*galK::spc, bgaA::*P*Zn-galT-2-sfgfp(iGEM)*, SpeR,TetR | This work |
| D39∆*galK* + pKB01-*galKgalT-2* | D39 ∆*galK::spc, bgaA::*P*Zn-galKgalT-2-sfgfp(iGEM)*, SpeR,TetR | This work |
| ***E. coli*** |  |  |
| DH5α | *F– Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169*rec*A1*end*A1*hsd*R17 (rK–, mK+)*pho*A*sup*E44 λ–*thi*-1*gyr*A96*rel*A1* | Invitrogen (b) |
| DH5α + pKB01 | AmpR | (b) |
| **Plasmids** |  |  |
| pORI138 | Ori+ repA-, deletion derivative of pWV01; SpeR | [1] |
| pKB01 | *bgaA,* P*zn-sfgfp(iGEM)+*, AmpR, TetR | [2] |
| pKB01-*galK* | *bgaA,* P*zn-galK-sfgfp(iGEM)*, AmpR, TetR | This work |
| pKB01-*lacD* | *bgaA,* P*zn-lacD-sfgfp(iGEM)*, AmpR, TetR | This work |
| pKB01-*manA* | *bgaA,* P*zn-manA-sfgfp(iGEM)*, AmpR, TetR | This work |
| pKB01-*nagA* | *bgaA,* P*ownnagA-nagA-sfgfp(iGEM)*, AmpR, TetR | This work |
| pKB01-*galT-2* | *bgaA,* P*zn-galT-2-sfgfp(iGEM)*, AmpR, TetR | This work |
| pKB01-*galKgalT-2* | *bgaA,* P*zn-galKgalT-2-sfgfp(iGEM)*, AmpR, TetR | This work |

SpeR, spectinomycin resistance marker, TmpR, trimethoprim resistance marker, AmpR, ampicillin resistance marker, TetR, tetracycline resistance marker.

**(a)** Laboratory stock obtained from the Department of Infection, Immunity & Inflammation, University of Leicester.

**(b)** Laboratory stock obtained from the Molecular Genetics Department, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Centre for Synthetic Biology, University of Groningen.

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