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| **Table S2. Strains and plasmids used in this study** | | |
| **Strain or plasmid** | **Description** | **Reference or source** |
| *E. coli* DC10B | Cloning strain | (Monk et al., 2012) |
| *E. coli* DH5α | Cloning strain | (Hanahan, 1983) |
| *S. aureus* RN4220 | *S. aureus* laboratory strain | (Kreiswirth et al., 1983) |
| *S. aureus* SEJ1 | ∆*spa* in RN4220 | (Gründling and Schneewind, 2007) |
| *S. aureus* WY110 | ∆*spa* ∆*sbi, sbi*::*ermB* in *S. aureus* SEJ1 | This work |
| *S. aureus* WY223 | P*spac-secA* in *S. aureus* RN4220 | This work |
| *S. aureus* WY230 | P*tet-secA:sfGFP* in *S. aureus* WY223 | This work |
| *S. aureus* ANG499 | P*spac-ltaS* in *S. aureus* RN4220 | (Gründling and Schneewind, 2007) |
| *S. aureus* WY418 | ∆*secDF* in *S. aureus* RN4220 | This work |
| *S. aureus* WY416 | ∆*rnd2* in *S. aureus* RN4220 | This work |
| *S. aureus* WY400 | ∆*rnd3* in *S. aureus* RN4220 | This work |
| *S. aureus* WY412 | ∆*secDF* ∆*rnd2*∆*rnd3* in *S. aureus* RN4220 | This work |
| pOS1 | *E. coli/S. aureus* shuttle vector | (Schneewind et al., 1993) |
| pSpAED | *spa* promoter, signal peptide and IgBDs E and D in pOS1 | This work |
| pSpAED/R10A | R10A variant of pSpAED | This work |
| pSpAED/S18L | S18L variant of of pSpAED | This work |
| pSpAED/ΔIA | ∆IA variant of pSpAED | This work |
| pCL55 | *S. aureus* integration vector | (Lee et al., 1991) |
| pCL55-SpA | Full length *spa* with its native promoter cloned in pCL55 | This work |
| pCL55-SpAR10A | R10A variant of pCL55-SpA | This work |
| pCL55-SpAS18L | S18L variant of pCL55-SpA | This work |
| pCL55-SpAΔIA | ∆IA variant of pCL55-SpA | This work |
| pCL55-SpASP-SasF | SpA signal peptide replaced by SasF signal peptide in pCL55-SpA | This work |
| pCL55-P*tet* | pCL55 with anhydrotetracycline inducible promoter | (Gründling and Schneewind, 2007) |
| pCL55-P*tet*-*secA:sfGFP* | SecA-sfGFP hybrid cloned into pCL55-P*tet* | This work |
| pMutin–HA | Single copy integration vector | Bacillus Genetic Stock Center |
| pMutin–HA-5’secA | *secA* promoter and 656 bp in pMutin-HA | This work |
| pKOR1-*secDF* | allelic replacement vector for *secDF* deletion | This work |
| pKOR1-*rnd2* | allelic replacement vector for *rnd2* deletion | This work |
| pSecDF | *secDF* ORF and 274 bp upstream in pOS1 | This work |