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| **Plasmid** | **Parent vector, relevant genotype and properties** | **Source or reference** |
| pET-28b(+) | T7 promoter, transcription start and terminator, His-tag, *lacI*, *aph,* pBR322 ORI; 5368 bp | Novagen, WI |
| pMAL-c5X | pMB1 origin, *lacI*, *malE*, *bla*, Factor Xa cleavage site; 5677 bp | NEB, MA |
| pCreSacB1 | p*groEL*-*cre*, oriE, oriM, *sacR*, *sacB*, *aph*;7891 bp | Dr. Steyn |
| pML523 | pUC origin, pAL5000ts origin; *sacB xylE loxP-gfp*2+m-*hyg*-*loxP*; 9,845 bp | (3) |
| pML1500 | pML523; *mmpS5*up *loxP*-*gfp*2+m-*hyg*-*loxP*; 10,797 bp | This study |
| pML1501 | pML1500; *loxP*-*gfp*2+m-*hyg*-*loxP* *mmpS5*down;11,662 bp | This study |
| pML1508 | pML523; *mmpS4*up *loxP*-*gfp*2+m-*hyg*-*loxP*; 11,212 bp | This study |
| pML1509 | pML1508; *loxP*-*gfp*2+m-*hyg*-*loxP* *mmpS4*down; 12,175 bp | This study |
| pML1342 | ColE1 origin; L5 *int*, *hyg, xylEm , loxP-*L5 *attP-lox*; *5,404 bp* | (4) |
| pML1544 | pML1342; *loxP-pnative-mmpS5-*L5 *attP-loxP*; 6,308 bp | This study |
| pML1545 | pML1342; *loxP-pnative-mmpS4-*L5 *attP-loxP*; 6,371 bp | This study |
| pML2300 | ColE1 origin*; xylEm, gfpm2+, aph*, Ms6 *int*, Ms6 *attP*; *6,821 bp* | (4) |
| pML1560 | ColE1 origin; *xylEm, aph*, *pnative-mmpS5,* Ms6 *int*, Ms6 *attP*; *6,688 bp* | This study |
| pML1561 | ColE1 origin; *xylEm, aph*, *pnative-mmpS4,* Ms6 *int*, Ms6 *attP*; *6,751 bp* | This study |
| pML1562 | ColE1 origin*, xylEm, aph*, Ms6 *int*, Ms6 *attP*; *5,746 bp* | This study |
| pML1565 | pML1500; *loxP*-*gfp*2+m-*hyg*-*loxP* *mmpL5*down;11,780 bp | This study |
| pML1566 | pML1508; *loxP*-*gfp*2+m-*hyg*-*loxP* *mmpL4*down; 12,293 bp | This study |
| pML1570 | pMAL-c5X; *malE*- *mmpS5e*; 6,096 bp | This study |
| pML1571 | pMAL-c5X; *malE*- *mmpS4e*; 6,090 bp | This study |
| pML1595 | pET-28b(+); *his-mmpS527-142*; 5,552 bp | This study |
| pML1596 | pET-28b(+); *his-mmps424-140*; 5,559 bp | This study |
| pML1801 | ColE1 origin, pAL5000 origin; hygR, *fxbA23*\_*gfp*2+m; 6,164 bp | (5) |
| pML1802 | pML1342; *loxP-fxbA23\_ gfp2+m*-L5 *attP-loxP*; 6,369 bp | This study |
| pML1816 | pML523; *mbtD*up *loxP*-*gfp*2+m-*hyg-loxP mbtD*down; 11, 825 bp | (3) |
| pML1828 | ColE1 origin, pAL5000 origin; *hyg, mbtG, HA tag –HIS tag*;6,853 bp | This study |

**Table S3. Plasmids used in this work.** Up- and downstream homologous sequences of the *mmpS5* and *mmpS4* genes are subscripted as up and down in pML1500 and pML1501 for *mmpS5* and in pML1508 and pML1509 for *mmpS4*. “Origin” means origin of replication. The genes *bla*, *hyg* and *aph* confer resistance to ampicillin, hygromycin and kanamycin, respectively. The L5 and Ms6 *attP* sites are required for site specific integration of plasmids into the chromosomal L5 and Ms6 *attB* sites by the mycobacteriophage L5 and Ms6 integrase *int* genes, respectively. The site-specific recombinase Cre excises DNA fragments that are flanked by *loxP* recognition sites. pAL5000ts denotes the temperature-sensitive origin of replication (6) of the pAL5000 plasmid (7). *E. coli* codon optimized *mmpS4* and *mmpS5* are designated *mmpS4e* and *mmpS5e*, respectively.The truncated *E. coli* codon optimized gene of *mmpS4* encoding an N-terminal 6xhis tag is designated *his-mmpS424-140*. The truncated *E. coli* codon optimized gene of *mmpS5* encoding an N-terminal 6xhis tag is designated *his-mmpS527-142*. The native promoters of *mmpS4* and *mmpS5* are designated *pnative-mmpS4* and *pnative-mmpS5*, respectively, and include approximately 500 bp of their respective upstream regions. The *sacS* gene of *B. subtilis* encodes the counterselective marker levansucrase that mediates sensitivity to sucrose (8). Its expression is regulated by SacR.