

Supplementary Table 1. Plasmid construction.

| Plasmid | Insert | Construction |
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| pAF022 | P_{spoIID} -yfp at <i>pyrD</i> | P_{spoIID} fragment (PY79; AFO063, 064) was digested with EcoRI & HindIII and ligated to pKM51. |
| pAF038 | P_{spank} - P_{spoVE} - <i>spoVE-gfp</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE-gfp</i> amplified (JDB1135; AFO100, 102) was digested with NheI & SphI and ligated to pDR111(D. Rudner). |
| pAF054 | P_{spoIIQ} -yfp at <i>sacA</i> | P_{spoIIQ} amplified (PY79; AFO126, 127) was digested with EcoRI/HindIII and ligated to pDG1662(2) cut with same. YFP cassette was digested with HindIII/BamHI and ligated to vector. P_{spoIIQ} -YFP was then digested with EcoRI/BamHI and ligated to pSac-Kan (6). |
| pAF078 | <i>ftsW</i> -yfp | <i>ftsW</i> amplified (PY79, AFO154, 155) was digested with EcoRI/XhoI and ligated to pKM67 |
| pAF083 | <i>tet</i> cassette at <i>sacA</i> | <i>tet</i> cassette amplified (pDG1515(3), AFO081, 082) was digested with EcoRI/SphI and ligated to pSac-Cm (6). |
| pAF100 | <i>spoVD-flag</i> | 3' <i>spoVD</i> -flag amplified (PY79, JDO763, AFO238), digested with EcoRI and SphI, ligated to pKL147(5). |
| pAF104 | P_{spoIID} - <i>cfp</i> at <i>sacA</i> | P_{spoIID} amplified (PY79; AFO063, 064) was digested with EcoRI/HindIII and ligated to pAF083. CFP cassette was then digested with HindIII/BamHI and ligated to vector. |
| pAF132 | addition of SpeI site | pDG1662(2) fragment amplified (AFO326, 327) digested with EcoRI/SphI and ligated to pDG1662(2). |
| pAF195 | <i>mls</i> at <i>sacA</i> | Mls cassette amplified (pDG1730(2); AFO458, 459), digested with EcoRI and SphI, ligated to pSac-Cm (6). |
| pAF211 | P_{spoVE} - <i>spoVE(G334A)</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE(G334A)</i> amplified (JDB1271, OJD006, AFO363) was digested with EcoRI/BamHI and ligated to pAF195 |
| pAF213 | P_{spoVE} - <i>spoVE(G343A)</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE(G343A)</i> amplified (JDB1355; OJD006, AFO363) was digested with EcoRI/BamHI and ligated to pAF195. |
| pAF215 | P_{spoVE} - <i>spoVE(E271A)</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE(E271A)</i> amplified (JDB1359; OJD006, AFO363) was digested with EcoRI/BamHI and ligated to pAF195. |
| pAF216 | P_{spoVE} - <i>spoVE(W69A)</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE(W69A)</i> amplified (JDB1850; OJD006, AFO363) was digested with EcoRI/BamHI and ligated to pAF195 |
| pAF239 | P_{spoVE} - <i>secY(1,6)-flag</i> at <i>amyE</i> | <i>secY</i> amplified (MG1655; AFO482, 509) digested with SpeI and BamHI, and ligated to pKM44 |
| pAF248 | P_{spoVE} - <i>spoVE</i> at <i>amyE</i> | P_{spoVE} - <i>spoVE</i> amplified (PY79; OJD006, AFO524) and digested with EcoRI/BamHI and ligated to |

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| | | pDG1662 |
| pAF250 | P_{spoVE} - <i>secY(1,6)</i> -flag at <i>sacA</i> | Fragment of pAF239 cut with EcoRI and BamHI ligated to pAF195 |
| pAF254 | P_{spoVE} - <i>spoVE</i> -GSGSGS- <i>spoVD</i> -flag at <i>amyE</i> | Linker- <i>spoVD</i> -flag amplified (PY79; AFO532, 536) digested with SpeI/BamHI and ligated to pAF248. |
| pAF257 | GSGSGS- <i>spoVD</i> -flag at <i>amyE</i> | linker- <i>spoVD</i> -flag amplified (PY79; AFO532, 536), digested with SpeI/BamHI and ligated to pAF248. |
| pAF260 | P_{spoVE} - <i>spoVE</i> (G334A)-GSGSGS- <i>spoVD</i> -flag at <i>amyE</i> | P_{spoVE} - <i>spoVE</i> (G334A) amplified (JDB1271; OJD006, AFO524) was digested with EcoRI/SpeI and ligated to pAF257 |
| pAF261 | P_{spoVE} - <i>spoVE</i> (G343A)-GSGSGS- <i>spoVD</i> -flag cm at <i>amyE</i> | P_{spoVE} - <i>spoVE</i> (G343A) amplified (JDB1355; OJD006, AFO524) digested with EcoRI/SpeI and ligated to pAF257 |
| pAF263 | P_{spoVE} - <i>spoVE</i> (E271A)-GSGSGS- <i>spoVD</i> -flag at <i>amyE</i> | P_{spoVE} - <i>spoVE</i> (E271A) amplified (JDB1357; OJD006, AFO524) was digested with EcoRI/SpeI and ligated to pAF257 |
| pAF273 | P_{spoVE} - <i>gfp</i> - <i>spoVD</i> (K496)-flag at <i>amyE</i> | Fragments amplified with (pKM61; OJD006, AFO553-r) and (pKM61; AFO553, 536) were sewn together, digested with EcoRI/BamHI and ligated to pDG1662(4) |
| pAF274 | P_{ftsW} - <i>ftsW</i> at <i>amyE</i> | P_{ftsW} - <i>ftsW</i> amplified (PY79; OJD199, AFO557), digested with EcoRI/BamHI and ligated to pDG1662(4) |
| pAF275 | P_{spoVE} - <i>spoVE</i> -GSGSGS- <i>pbpB</i> -flag at <i>amyE</i> | <i>pbpB</i> amplified (PY79; AFO555, 556), digested with SpeI/BamHI and ligated to pAF248 |
| pAF276 | P_{ftsW} - <i>ftsW</i> -GSGSGS- <i>pbpB</i> -flag at <i>amyE</i> | <i>pbpB</i> amplified (PY79; AFO555, 556), digested with SpeI/BamHI and ligated to pAF274 |
| pAF278 | P_{spoVE} - <i>spoVE</i> -GSGSGS- <i>spoVD</i> (K496A)-flag at <i>amyE</i> | <i>spoVD</i> (K496A) amplified (pAF273; AFO532, 536) digested with SpeI/BamHI and ligated to pAF248. |
| pAF310 | P_{spoVE} - <i>spoVE</i> -GSGSGS- <i>spoVD</i> (Q227E)-flag at <i>amyE</i> | <i>spoVD</i> (Q227E) amplified (pAF317; AFO532, 536) was digested with SpeI/BamHI and ligated to pAF248 |
| pAF317 | P_{spoVE} - <i>gfp</i> - <i>spoVD</i> (Q227E) flag at <i>amyE</i> | Fragments (pKM61; OJD006, AFO593-r; AFO593, 536) sewn together, digested with EcoRI/BamHI and ligated to pDG1662(4) |
| pAF328 | 3' <i>spoVD</i> -mCherry | mCherry amplified (ODJ184, 185) was digested with XhoI/SphI and ligated to pKM5 |
| pAF402 | 3' <i>spoVD</i> (K496A)-mCherry | 3' <i>spoVD</i> amplified (pAF273; OJD763, OJD764) was digested with EcoRI/XhoI and ligated to pAF328 cut with same |
| pKM5 | 3' <i>spoVD</i> - <i>gfp</i> | 3' <i>spoVD</i> amplified (PY79; OJD763, OJD764) was digested with EcoRI/XhoI and ligated to pKL147(5) |
| pKM51 | <i>yfp</i> at <i>pyrD</i> | <i>yfp</i> amplified (<i>yfp</i> cassette; JD643, 666) was digested with HindIII/BamHI and ligated to pPyr-Cm(6) |
| pKM57 | P_{spoVE} - <i>gfp</i> at <i>amyE</i> | <i>yfp</i> amplified (<i>yfp</i> cassette; OJD087, 088) was |

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| | | digested with HindIII/BamHI and ligated to pKM44 |
| pKM61 | P_{spoVE} - <i>gfp-spoVD</i> at <i>amyE</i> | <i>spoVD</i> amplified (PY79; OJD098, 099) was digested with SpeI and ligated to pKM57. |
| pKM65 | <i>cfp</i> | <i>cfp</i> amplified (<i>cfp</i> cassette; OJD152, 153) was digested with XhoI/SphI and ligated to pKL147(5) |
| pKM67 | <i>yfp</i> | <i>yfp</i> amplified (<i>yfp</i> cassette; OJD154, 155) was digested with XhoI/SphI and ligated to pKL147(5) |
| pKM77 | P_{spoVE} - <i>yfp</i> at <i>amyE</i> | <i>yfp</i> amplified (<i>yfp</i> cassette; OJD087,088) digested with HindIII/BamHI and ligated to pKM44 (8) |
| pKM80 | P_{spoVE} - <i>yfp-spoVD</i> at <i>amyE</i> | <i>spoVD</i> amplified (PY79; OJD098, 099) was digested with SpeI and ligated to pKM77 |
| pMR14 | P_{spoVE} - <i>yfp-spoVD</i> at <i>gltA</i> | pKM80 digested with EcoRI/BamHI and P_{spoVE} - <i>yfp-spoVD</i> fragment ligated to pGlt-Cm(6) |
| pMR9 | P_{spoVE} - <i>spoVE-cfp</i> at <i>sacA</i> | P_{spoVE} - <i>spoVE-cfp</i> (JDB1376, OJD006, JD643) digests with EcoRI/BamHI and ligated to pSac-tet |
| pMR12 | P_{spoVE} - <i>ftsW-yfp</i> at <i>amyE</i> | <i>ftsW-yfp</i> (JDB1559, AFO054 JD643) digested with SpeI/BamHI and ligated to pKM44(8) cut with same |
| pMR14 | P_{spoVE} - <i>yfp-spoVD</i> at <i>gltA</i> | pKM80 digested with EcoRI/BamHI and P_{spoVE} - <i>yfp-spoVD</i> fragment ligated to pGlt-Cm(6) |
| pMR35 | P_{spoVE} - <i>yfp-cfp</i> at <i>amyE</i> | <i>Cfp</i> amplified (OPM100, OPM101) digested with SpeI and ligated to pKM77 cut with NheI |

For *E.coli* replication

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| pAF172 | P_{ara} -GST | RBS-GST-Xa cut from pALEX(7) with XbaI/SalI was ligated to to pBAD24(4) cut with NheI/SalI |
| pAF175 | P_{ara} -GST- <i>spoVD</i> AmpR | <i>spoVD</i> amplified PY79; AFO437, 426) was digested with EcoRI/SalI then ligated to pAF172. |
| pAF186 | T7-6his- <i>spoVE-gfp</i> | <i>spoVE-gfp</i> amplified (JDB1134; AFO439,389) was digested BamHI/PstI and ligated to pETDuet-1 (Novagen) digested with BglII/PstI. |
| pAF188 | P_{ara} -6his- <i>spoVE-gfp</i> | 6his- <i>spoVE-gfp</i> fragment from pAF186 digested with XbaI/PstI ligated to pBAD33(4). |
| pAF190 | P_{ara} -10xhis | ligated oligos into pBAD24(4) |
| pAF191 | P_{ara} -10xHis- <i>spoVE</i> -GFP | <i>spoVE-gfp</i> amplified (PY79; AFO439, 389) was digested BamHI/PstI and ligated into pAF190 digested with BglII/PstI. |
| pAF231 | P_{ara} -GST- <i>spoVD</i> | Fragment from digesting pAF175 with BstEII/SalI was ligated to pBAD33(4) cut with same |
| pAF232 | P_{ara} -GST | Fragment from digesting pAF172 with BstEII/PstI was ligated to pBAD33(4) cut with same |
| pAF233 | P_{ara} -10xhis- <i>gfp</i> | <i>gfp</i> amplified (pKL147(5); AF)438, 439) was digested BamHI/PstI and ligated to pAF190 digested with BglII/PstI. |

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| pAF253 | P_{ara} -10his-ftsW-gfp | ftsW-gfp amplified (JDB223; AFO423, 439) was digested with BamHI/PstI and ligated into pAF190 cut with BglII/PstI. |
| pAF256 | P_{ara} -10xhis-ftsW-gfp | 10xhis-ftsW-gfp from pAF253 digested with XbaI/PstI ligated to pBAD33(4) |
| pAF267 | P_{ara} -GST-Pbp2B | pbpB amplified (PY79; AFO534 538) was digested with Sall/SphI and ligated to pAF172 |

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