

Dataset 1: Plasmids used and generated in this study. Unless indicated otherwise, genomic DNA of *S. venezuelae* was used for PCR amplification.

Name	Description	Construction/Reference
pMS82	Plasmid cloning vector for the conjugal transfer of DNA from <i>E. coli</i> to <i>Streptomyces</i> spp. Integrates site specifically at the $\Phi BT1$ attachment site (Hyg <sup>R</sup> )	(1)
pKF280	Vector for the construction of gene fusions to <i>ypet</i> , based on pIJ6902 but with the <i>tipA</i> promoter removed. Integrates at the $\phi C31$ attachment site (Apr <sup>R</sup> )	(2)
pKF351	Derivative of pKF280 encoding the FtsZ-YPet fusion	(2)
pUZ8002	RP4 derivative with non-transmissible <i>oriT</i> , (Kan <sup>R</sup> )	(3)
pIJ773  pIJ773 (continued)	pBluescript KS (+) containing the apramycin resistance gene <i>apr</i> and <i>oriT</i> of plasmid RP4, flanked by FRT sites (Apr <sup>R</sup> ). Used as template for the amplification of the <i>apr oriT</i> cassette for 'Redirect' PCR-targeting	(4)
pIJ790	Modified $\lambda$ RED recombination plasmid [ <i>oriR101</i> ] [ <i>repA101(ts)</i> ] <i>araBp-gam-bet-exo</i> (Cam <sup>R</sup> )	(4)
pIJ2742	Temperature sensitive plasmid (pGM1190-based) encoding the I-SceI Meganuclease (Thio <sup>R</sup> /Apr <sup>R</sup> )	(5)
pIJ10257	Plasmid cloning vector for the conjugal transfer of DNA (under control of the <i>ermE</i> * constitutive promoter) from <i>E. coli</i> to <i>Streptomyces</i> spp. Integrates specifically at the $\Phi BT1$ attachment site (Hyg <sup>R</sup> )	(6)
pIJ10754	pUC19 derivatate for the construction of C-Terminal 3xFLAG-gene fusions (Carb <sup>R</sup> )	Chris den Hengst, unpublished
pIJ12738	Delivery vector (containing the I-SceI recognition site) required for I-SceI-mediated gene deletions	(5)
pKT25	Two-hybrid plasmid, N-terminal <i>cyaAT25</i> fusion (Kan <sup>R</sup> )	(7)
pKNT25	Two-hybrid plasmid, C-terminal <i>cyaAT25</i> fusion (Kan <sup>R</sup> )	(7)
pUT18	Two-hybrid plasmid, C-terminal <i>cyaAT18</i> fusion (Amp <sup>R</sup> )	(7)

Name	Description	Construction/Reference
pUT18C	Two-hybrid plasmid, N-terminal <i>cyaAT18</i> fusion (Amp <sup>r</sup> )	(7)
pGBT9	Yeast two-hybrid cloning vector, <i>GAL4 DNA-BD, TRP1</i> , (Amp <sup>R</sup> )	Clontech
pGAD424	Yeast two-hybrid cloning vector, <i>GAL4 AD, LEU2</i> , (Amp <sup>R</sup> )	Clontech
pGADT7	Yeast two-hybrid Gateway vector, (Amp <sup>R</sup> )	Clontech
pDON207	Gateway entry vector (Gent <sup>R</sup> )	Life Technologies
pTYP12	Expression vector with a cleavable N-terminal Intein tag (Amp <sup>R</sup> )	New England Biolabs
pKF304	<i>dynAB</i> in pKF371 (Hyg <sup>R</sup> )	<i>dynAB</i> locus was amplified with oligo KF1115 and KF1116 followed by restriction digestion with Acc651 and NsiI and ligated into pKF371 cut with Acc651 and NsiI.
pKF351	Derivative of pKF280 encoding the FtsZ-YPet fusion (Apr <sup>R</sup> )	<i>ftsZ</i> including promotor region was amplified with oligo KF742 and KF956 followed by restriction digestion with XbaI and NdeI and ligation into pKF280 cut with XbaI and NdeI.
pKF335	<i>dynAB-ypet</i> in pKF304 (Hyg <sup>R</sup> )	<i>ypet</i> was amplified from pKF280 with oligos KF1119 and KF1120 which add a 6x Gly/Ser linker to the N-terminus of YPet. The <i>ypet</i> PCR product was digested with BamHI and NsiI and ligated into pKF304 cut with BamHI and NsiI.
pKF337	<i>dynAB</i> in pKF304 (Hyg <sup>R</sup> )	<i>dynAB</i> was amplified using oligo KF1114 and KF1116 followed by restriction digestion with NsiI and Acc651 and ligation into pKF371 cut with NsiI and Acc651.
pKF371	Derivative of pMS82 (Hyg <sup>R</sup> )	pMS82 was amplified with oligos KF1117 and KF1118 followed by relegation to delete one BamHI site.
pIJ10750	pMS82 with an extended Multiple Cloning Site (MCS), (Hyg <sup>R</sup> )	Oligo 23 and 24 were phosphorylated with PNK, annealed, and inserted into pMS82 cut with AvrII and KpnI.
pIJ10770	Modified pIJ10750 lacking an intrinsic apramycin promoter upstream of the extended multiple cloning site (Hyg <sup>R</sup> )	pIJ10750 was amplified with oligos 295 and 296 followed by restriction digestion with HindIII and relegation.
pSS1	pTYP12 with <i>dynA</i> (Carb <sup>R</sup> )	<i>dynA</i> was amplified with oligo 19 and 20 followed by restriction digestion with NdeI and EcoRI and ligation into pTYB12 cut with NdeI and EcoRI.
pSS3	pIJ10257 carrying <i>ftsZ</i> (Hyg <sup>R</sup> )	<i>ftsZ</i> was amplified with oligos <i>ftsZ_pIJ10257_FWD/REV</i> followed by restriction digestion with NdeI and HindIII and ligation into pIJ10257 cut with NdeI and HindIII.
pSS5	<i>P<sub>ftsZ</sub>ftsZ-ypet</i> in pIJ10750 (Hyg <sup>R</sup> )	The fragment containing <i>P<sub>ftsZ</sub>ftsZ-ypet</i> was liberated from pKF351 by restriction digestion with BamHI and KpnI, followed by treatment with T4 DNA polymerase and ligation into the EcoRV site of pMS82.

Name	Description	Construction/Reference
pSS6	<i>dynB</i> in pTYP12 (Amp <sup>R</sup> )	<i>dynB</i> was amplified with oligo 21 and 22 followed by restriction digestion with NdeI and EcoRI and ligation into pTYB12 cut with NdeI and EcoRI.
pSS8	<i>mcherry</i> in pIJ10750 (Hyg <sup>R</sup> )	Insertion of codon-optimised <i>mcherry</i> between the XhoI and KpnI site of pIJ10750.
pSS12	<i>P<sub>dyn</sub>-dynA-mcherry</i> in pSS8 (Hyg <sup>R</sup> )	<i>P<sub>dyn</sub>-dynA</i> was amplified with oligos 29 and 30 followed by restriction digestion with HindIII and AvrII and ligation into pSS8 cut with HindIII and AvrII.
pSS33	<i>dynA</i> in pUT18C (Amp <sup>R</sup> )	<i>dynA</i> was amplified with oligo 65 and 66 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI.
pSS18	<i>dynA</i> in pET18b (Carb <sup>R</sup> )	<i>dynA</i> was isolated from pSS1 by restriction digestion with NdeI and BamHI and ligated into pET15b cut with NdeI and BamHI.
pSS37	StrepII- <i>dynB</i> in pETDuet-1(Carb <sup>R</sup> )	<i>dynB</i> was amplified with oligo 12 and 13, adding an N-terminal StrepII-tag. The PCR product was digested with NdeI and KpnI and ligated into pETDuet-1 cut with NdeI and KpnI.
pSS39	<i>dynAB</i> in pET15b (Carb <sup>R</sup> )	<i>dynAB</i> fragment was liberated from pSS37 by restriction digestion with AscI and HindIII and ligated into pSS18 cut with AscI and HindIII.
pSS40	<i>dynA</i> [K74A] in pTYP12 (Carb <sup>R</sup> )	Mutagenesis of <i>dynA</i> to introduce K74A mutation: amplification of <i>dynA</i> with oligo pair Int-fw/71 and 72/66 using pSS1 as template. The two PCR fragments were fused by overlap-extension PCR with oligo Int-fw and 66 followed by restriction digestion with NdeI and EcoRI and ligation into pTYP12 cut with NdeI and EcoRI.
pSS41	<i>dynB</i> [K129A] in pTYB12 (Carb <sup>R</sup> )	Mutagenesis of <i>dynB</i> to introduce K129A mutation: amplification of <i>dynB</i> with oligo pair Int-fw/73 and 74/70 using pSS6 as template. The two PCR fragments were fused by overlap-extension PCR with oligo Int-fw and 70 followed by restriction digestion with NdeI and EcoRI and ligation into pTYP12 cut with NdeI and EcoRI.
pSS44	<i>dynB</i> in pKNT25 (Kan <sup>r</sup> )	<i>dynB</i> was amplified with oligo 67 and 68 followed by restriction digestion with BamHI and HindIII and ligation into pKNT25 cut with BamHI and HindIII.
pSS47	<i>dynAB</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynAB</i> was liberated from pSS39 by restriction digestion with NdeI and HindIII and ligated into pIJ10257 cut with NdeI and HindIII.
pSS49	<i>dynA</i> in pIJ10502 (Carb <sup>R</sup> )	<i>dynA</i> was amplified with oligo 107 and 108 followed by restriction digestion with KpnI and XhoI and ligation into pIJ10502 cut with KpnI and XhoI.
pSS50	pIJ10754 with <i>dynB</i> (Carb <sup>R</sup> )	<i>dynB</i> was amplified with oligo 105 and 106 followed by restriction digestion with KpnI and XhoI and ligation into pIJ10502 cut with KpnI and XhoI.

Name	Description	Construction/Reference
pSS51	<i>dynB</i> in pUT18 (Amp <sup>R</sup> )	<i>dynB</i> was amplified with oligo 69 and 70 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18 cut with BamHI and EcoRI.
pSS53	<i>dynB-3xFLAG</i> in pKF337 (Hyg <sup>R</sup> )	<i>dynB-3xFLAG</i> was isolated from pSS50 by restriction digestion with BamHI and AsiSI and ligated into pKF377 cut with BamHI and AsiSI.
pSS61	pKF351 with <i>ypet</i> replaced by <i>mturquoise2</i> (Apr <sup>R</sup> )	Codon-optimised <i>mturquoise 2</i> was amplified with oligo 112 and 113 followed by restriction digestion with NdeI and BglII and ligation of into pKF351 cut with NdeI and BglII to replace <i>ypet</i> with <i>mturquoise2</i> .
pSS84	pIJ10257-leaderless <i>ermE*</i> promoter with <i>dynA-dynB</i> (Hyg <sup>R</sup> )	The <i>P<sub>ermE*</sub></i> promoter was amplified with oligo 159 and 160 using pIJ10257 as template; <i>dynA</i> was amplified with oligo 161 and 162 and both PCR products were fused by PCR using oligo 159 and 162 followed by restriction digestion with KpnI and AscI and ligation into pSS47 cut with KpnI and AscI.
pSS87	<i>dynB-mcherry</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynB</i> was amplified with oligo 21 and 32 followed by restriction digestion with NdeI and AvrII and ligation into pSS88 cut with NdeI and AvrII.
pSS88	<i>mcherry</i> pIJ10257 (Hyg <sup>R</sup> )	Codon-optimised <i>mcherry</i> was amplified with oligo 82-fw and 82-rev followed by restriction digestion with NdeI and KpnI and ligation pIJ10257 partially digested with NdeI and KpnI.
pSS89	<i>dynB-ypet</i> in pKF337 (Hyg <sup>R</sup> )	<i>dynA-dynB</i> was liberated from pKF335 by restriction digestion with AscI and AvrII and ligated into pKF337 cut with AscI and AvrII.
pSS91	<i>dynA-3xFLAG</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynA-3xFLAG</i> fragment was isolated from pSS49 by restriction digestion with AscI and HindIII and ligated into pSS84 cut with AscI and HindIII.
pSS100	<i>dynB</i> [K129A] in pKT25 (Kan <sup>R</sup> )	<i>dynB</i> [K129A] was amplified with oligo 69 and 70 using pSS6 as template followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI.
pSS101	<i>dynB</i> [K129A] in pKNT25 (Kan <sup>R</sup> )	<i>dynB</i> [K129A] was amplified with oligo 67 and 68 using pSS41 as template followed by restriction digestion with BamHI and HindIII and ligation into pKNT25 cut with BamHI and HindIII.
pSS102	<i>dynA</i> [K74A] in pUT18 (Amp <sup>R</sup> )	<i>dynA</i> [K74A] was amplified with oligo 63 and 64 using pSS40 as template followed by restriction digestion with BamHI and HindIII and ligation into pUT18 cut with BamHI and HindIII.
pSS103	<i>dynA</i> [K74A] in pKNT25 (Kan <sup>R</sup> )	<i>dynA</i> [K74A] was amplified with oligo 63 and 64 using pSS40 as template followed by restriction digestion with BamHI and HindIII and ligation into pKNT25 cut with BamHI and HindIII.

Name	Description	Construction/Reference
pSS104	<i>dynA</i> [K74A] in pKT25 (Kan <sup>R</sup> )	<i>dynA</i> [K74A] was amplified with oligo 65 and 66 using pSS40 as template followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI.
pSS105	<i>dynB</i> in pKT25 (Kan <sup>R</sup> )	<i>dynB</i> was amplified with oligo 69 and 70 followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI.
pSS107	pIJ12738 carrying $\Delta$ <i>dynA</i> including 2-kb flanking sequence (Apr <sup>R</sup> )	1) Amplification of $\Delta$ <i>dynA</i> including ~2kb upstream sequence using oligo 179 and 191 followed by restriction digestion with HindIII and SpeI; 2) restriction digestion of PCR product with SpeI+KpnI and insertion into pSS110 cut with SpeI+KpnI; 3) Insertion of digested PCR product into pIJ12738 cut with HindIII and SpeI; 4) Amplification of $\Delta$ <i>dynA</i> including 2kb downstream sequence using oligo 182 and 192 followed by restriction into SpeI and KpnI; 5) Insertion of digested PCR product into SpeI-KpnI site of pIJ12738 containing the D <i>dynA</i> -upstream.
pSS115	<i>dynB</i> in pUT18C (Amp <sup>R</sup> )	<i>dynB</i> was amplified with oligo 69 and 70 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI.
pSS116	<i>dynB</i> [K129A] in pUT18C (Amp <sup>R</sup> )	<i>dynB</i> [K129A] was amplified with oligo 69 and 70 using pSS6 as template followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI.
pSS117	<i>dynA</i> [K74A] in pUT18C (Amp <sup>R</sup> )	<i>dynA</i> [K74A] was amplified with oligo 65 and 66 using pSS40 as template followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI
pSS118	<i>dynB</i> [K129A] in pUT18 (Amp <sup>R</sup> )	<i>dynB</i> [K129A] was amplified with oligo 67 and 68 using pSS41 as template followed by restriction digestion with BamHI and HindIII and ligation into pUT18 cut with BamHI and HindIII
pSS129	<i>dynB</i> [K129A]- <i>ypet</i> in pSS89 (Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS118 by restriction digestion with AsisSi+MluI followed by ligation into pSS89 cut with AsiSI+MluI.
pSS130	<i>dynA-dynB-3xFLAG</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynAB-3xFLAG</i> fragment was isolated from pSS53 by restriction digestion with AscI and AvrII followed by ligation into pSS84 cut with AscI and AvrII.
pSS132	<i>dynA</i> [K74A] in pKF337(Hyg <sup>R</sup> )	<i>dynA</i> [K74A] was isolated from pSS102 by restriction digestion with BbvCI and AscI followed by ligation into pKF377 cut with BbvCI and AscI.
pSS134	<i>dynA-dynB-ypet</i> in pSS84 (Hyg <sup>R</sup> )	<i>dynA-dynB-ypet</i> fragment was isolated from pSS84 by restriction digestion with AscI and AvrII followed by ligation into pSS84 cut with AscI and AvrII.

Name	Description	Construction/Reference
pSS135	<i>dynB</i> [K129A] in pSS132 (Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS118 by restriction digestion with AsiSI and MluI followed by ligation into pSS132 cut with MluI and AsiSI.
pSS141	<i>dynA-dynB</i> [K129A]- <i>ypet</i> in pSS134 (Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS129 by sequential digestion with BbvCI and MluI and inserted into pSS134
pSS142	<i>dynA</i> [K74A]- <i>dynB</i> [K129A]- <i>ypet</i> in pSS134 (Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS135 by sequential digestion with BbvCI and MluI and inserted into pSS134
pSS149	pIJ10257 with <i>ypet</i> (Hyg <sup>R</sup> )	Codon-optimised <i>ypet</i> was amplified with oligo 270 and 271 followed by restriction digestion with NdeI and XhoI and ligated into pIJ10257 cut with NdeI and XhoI.
pSS172	pIJ10770 carrying <i>mcherry</i> for the construction of C-terminal fluorescent gene fusions (Hyg <sup>R</sup> )	Insertion of codon-optimised <i>mcherry</i> between the XhoI and KpnI site of pIJ10770.
pSS178	pIJ10770 with <i>P<sub>dyn</sub>-mcherry</i> (Hyg <sup>R</sup> )	Overlap extension PCR to fuse dynamin promoter to <i>mcherry</i> ; 1) Amplification of the dynamin promoter fragment with oligo 304 and 305; 2) Amplification of <i>mcherry</i> with 308 and 309; 3) Fusion PCR using oligo 304 and 309 followed by restriction digestion with HindIII and XhoI; 4) Digested <i>P<sub>dyn</sub>-mcherry</i> fragment ligated into pIJ10770 cut with HindIII and XhoI.
pSS180	pSS89 with <i>ypet</i> replaced by <i>mcherry</i> (Hyg <sup>R</sup> )	Amplification of codon-optimised <i>mcherry</i> with oligo 332 and 333, introducing an 8AA N-terminal linker (MHGSGSGS), followed by restriction digestion with NsiI and BamHI and ligation into pSS89 cut with NsiI and BamHI.
pSS188	<i>P<sub>dyn</sub>-mcherry-dynA</i> in pIJ10770 (Hyg <sup>R</sup> )	Amplification of <i>dynA</i> using oligo 310 and 311 followed by restriction digestion with XhoI and Asp718 and ligation into pSS178 cut with XhoI and Asp718.
pSS192	<i>P<sub>dyn</sub>-mcherry-dynA-dynB-ypet</i> in pIJ10770 (Hyg <sup>R</sup> )	1) Isolation of <i>dynB-ypet</i> fragment from pKF355 by restriction digestion with BamHI, ends were blunted and DNA was digested with AscI. 2) Ligation of the <i>dynB-ypet</i> fragment in pSS188 which was first cut with KpnI, blunted and then cut with AscI.
pSS196	<i>P<sub>sepF</sub>-sepF-mcherry</i> in pSS172 (Hyg <sup>R</sup> )	Amplification of <i>sepF</i> , including promoter region, using oligo 336 and 337 followed by restriction digestion with HindIII and AvrII; ligation of digested PCR fragment into pSS172 cut with HindIII and AvrII.
pSS208	<i>dynA</i> [K74A]- <i>dynB-ypet</i> in pSS134 (Hyg <sup>R</sup> )	<i>dynA</i> [K74A] was isolated from pSS142 by restriction digestion with BbvCI and AscI followed by ligation into pSS134 cut with BbvCI and AscI.
pSS219	<i>sepF2</i> in pIJ10770 (Hyg <sup>R</sup> )	Amplification of <i>sepF2</i> with oligo 396 and 397 followed by restriction digestion with XhoI and EcoRV and ligation into pIJ10770 which was

Name	Description	Construction/Reference
pSS219 (continued)		cut with KpnI, blunted and subsequently digested with XhoI.
pSS220	<i>sepF2</i> in pKT25 (Kan <sup>R</sup> )	<i>sepF2</i> was amplified with oligo 404 and 405 followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI
pSS221	<i>sepF3</i> in pKT25 (Kan <sup>R</sup> )	<i>sepF3</i> was amplified with oligo 398 and 399 followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI
pSS222	<i>sepF</i> in pKT25 (Kan <sup>R</sup> )	<i>sepF</i> was amplified with oligo 401 and 402 followed by restriction digestion with BamHI and EcoRI and ligation into pKT25 cut with BamHI and EcoRI
pSS223	<i>sepF2</i> in pUT18 (Amp <sup>R</sup> )	<i>sepF2</i> was amplified with oligo 404 and 405 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18 cut with BamHI and EcoRI
pSS224	<i>sepF3</i> in pUT18 (Amp <sup>R</sup> )	<i>sepF3</i> was amplified with oligo 398 and 399 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18 cut with BamHI and EcoRI
pSS225	<i>sepF</i> in pUT18 (Amp <sup>R</sup> )	<i>sepF</i> was amplified with oligo 401 and 402 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18 cut with BamHI and EcoRI
pSS227	<i>sepF3</i> in pKTN25 (Kan <sup>R</sup> )	<i>sepF3</i> was amplified with oligo 398 and 400 followed by restriction digestion with BamHI and EcoRI and ligation into pKTN25 cut with BamHI and EcoRI
pSS228	<i>sepF</i> in pKTN25 (Kan <sup>R</sup> )	<i>sepF</i> was amplified with oligo 401 and 403 followed by restriction digestion with BamHI and EcoRI and ligation into pKTN25 cut with BamHI and EcoRI
pSS229	<i>sepF2</i> in pKTN25 (Kan <sup>R</sup> )	<i>sepF2</i> was amplified with oligo 404 and 406 followed by restriction digestion with BamHI and EcoRI and ligation into pKTN25 cut with BamHI and EcoRI
pSS231	<i>sepF3</i> in pUT18C (Amp <sup>R</sup> )	<i>sepF3</i> was amplified with oligo 398 and 400 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI
pSS232	<i>sepF</i> in pUT18C (Amp <sup>R</sup> )	<i>sepF</i> was amplified with oligo 401 and 403 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI
pSS233	<i>sepF2</i> in pUT18C (Amp <sup>R</sup> )	<i>sepF2</i> was amplified with oligo 404 and 406 followed by restriction digestion with BamHI and EcoRI and ligation into pUT18C cut with BamHI and EcoRI.
pSS234	<i>P<sub>sepF2</sub>-mcherry-sepF2</i> in pIJ10770 (Hyg <sup>R</sup> )	Overlap extension PCR to fuse <i>sepF2</i> promoter to <i>mcherry</i> : Amplification of the <i>sepF2</i> promoter fragment with oligo 394 and 338 and

Name	Description	Construction/Reference
pSS234 (continued)		amplification of <i>mcherry</i> with 395 and 309. The two PCR fragments were fused by PCR using oligo 338 and 309 followed by restriction digestion with HindIII and XhoI. The digested <i>P<sub>dyn</sub>-mcherry</i> fragment was then ligated into pSS219 cut with HindIII and XhoI.
pSS249	<i>sepF2</i> in pDONR207 (Gent <sup>R</sup> )	<i>sepF2</i> was amplified with oligo 442 and 443 and inserted into the Gateway TOPO entry vector pDONR207 via BP recombination.
pSS250	<i>sepF2</i> in pGADT7 (Amp <sup>R</sup> )	<i>sepF2</i> was moved from pSS249 into the gateway compatible destination vector pGADT7 via LR recombination.
pSS251	<i>sepF2</i> in pGBKT7 (Amp <sup>R</sup> )	<i>sepF2</i> was moved from pSS249 into the gateway compatible destination vector pGBKT7 via LR recombination.
pSS253	<i>ftsZ</i> in pGBT9 (Amp <sup>R</sup> )	<i>ftsZ</i> was amplified with oligo 449 and 450 followed by restriction digestion with EcoRI and PstI and ligation into pGBT9 cut with EcoRI and PstI.
pSS254	<i>ftsZ</i> in pGAD424 (Amp <sup>R</sup> )	<i>ftsZ</i> was amplified with oligo 449 and 450 followed by restriction digestion with EcoRI and PstI and ligation into pGAD424 cut with EcoRI and PstI.
pSS257	<i>dynA</i> in pDON207 (Gent <sup>R</sup> )	<i>dynA</i> was amplified with oligo 464 and 465 and inserted into the Gateway entry vector pDONR207 via BP recombination.
pSS259	<i>dynB</i> in pDON207 (Gent <sup>R</sup> )	<i>dynB</i> was amplified with oligo 467 and 468 and inserted into the Gateway entry vector pDONR207 via BP recombination.
pSS261	<i>sepF3</i> in pDON207 (Gent <sup>R</sup> )	<i>sepF3</i> was amplified with oligo 476 and 477 and inserted into the Gateway entry vector pDON207 via BP recombination.
pSS264	<i>sepF</i> in pDONR207 (Gent <sup>R</sup> )	<i>sepF</i> was amplified with oligo 473 and 474 and inserted into the Gateway entry vector pDON207 via BP recombination.
pSS269	<i>dynA</i> in pGADT7 (Amp <sup>R</sup> )	<i>dynA</i> was moved from pSS257 into the gateway compatible destination vector pGADT7 via LR recombination.
pSS270	<i>dynB</i> in pGADT7 (Amp <sup>R</sup> )	<i>dynB</i> was moved from pSS259 into the gateway compatible destination vector pGADT7 via LR recombination.
pSS271	<i>sepF</i> in pGADT7 (Amp <sup>R</sup> )	<i>sepF</i> was moved from pSS261 into the gateway compatible destination vector pGADT7 via LR recombination.
pSS272	<i>sepF3</i> in pGADT7 (Amp <sup>R</sup> )	<i>sepF3</i> was moved from pSS264 into the gateway compatible destination vector pGADT7 via LR recombination.
pSS273	<i>dynB</i> in pGBKT7 (Amp <sup>R</sup> )	<i>dynB</i> was moved from pSS259 into the gateway compatible destination vector pGBKT7 via LR recombination.
pSS274	<i>dynA</i> in pGBKT7 (Amp <sup>R</sup> )	<i>dynA</i> was moved from pSS257 into the gateway compatible destination vector pGBKT7 via LR recombination.



Name	Description	Construction/Reference
pSS275	<i>sepF</i> in pGBKT7 (Amp <sup>R</sup> )	<i>sepF</i> was moved from pSS264 into the gateway compatible destination vector pGBKT7 via LR recombination.
pSS276	<i>sepF3</i> in pGBKT7 (Amp <sup>R</sup> )	<i>sepF3</i> was moved from pSS261 into the gateway compatible destination vector pGBKT7 via LR recombination.
pSS307	<i>ypet-dynA</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynA</i> was amplified with oligo 98 and 310 followed by restriction digestion with XhoI and AvrII and ligation into pSS149 cut with XhoI and AvrII.
pSS308	<i>ypet-dynA</i> [K74A] in pIJ10257 (Hyg <sup>R</sup> )	<i>dynA</i> [K74A] was amplified from pSS102 with oligo 98 and 310 followed by restriction digestion with XhoI and AvrII and ligation into pSS149 cut with XhoI and AvrII.
pSS310	<i>ypet-dynA-dynB</i> in pIJ10557(Hyg <sup>R</sup> )	<i>dynB</i> was isolated from pSS84 by restriction digestion with AscI and AvrII and ligated into pSS307 cut with AscI and AvrII.
pSS311	<i>ypet-dynA-dynB</i> [K129A] in pIJ10257(Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS84 by restriction digestion with AscI and AvrII and ligated into pSS307 cut with AscI and AvrII
pSS316	<i>ypet-dynA</i> [K74A]- <i>dynB</i> in pIJ10257 (Hyg <sup>R</sup> )	<i>dynB</i> was isolated from pSS84 by restriction digestion with AscI and AvrII and ligated into pSS308 cut with AscI and AvrII.
pSS317	<i>ypet-dynA</i> [K74A]- <i>dynB</i> [K129A] in pIJ10257 (Hyg <sup>R</sup> )	<i>dynB</i> [K129A] was isolated from pSS84 by restriction digestion with AscI and AvrII and ligated into pSS308 cut with AscI and AvrII.
pSS353	<i>dynA-dynB</i> $\Delta$ TM- <i>ypet</i> in pSS84 (Hyg <sup>R</sup> )	Removal of the two transmembrane segments in <i>dynB</i> and insertion of a 15AA linker (LEGSGQGPGSGQGSQ). The two <i>dynB</i> fragments resulting from PCR amplification with oligo pair 619/620 and 621/622 were fused by PCR with oligo 619 and 622 followed by restriction digestion with AscI and AvrII and ligation into pSS84 cut with AscI and AvrII.
pMB169	<i>ssgA</i> in pKNT25 (Kan <sup>R</sup> )	<i>ssgA</i> was amplified with oligo <i>ssgA</i> -BACTH F/R followed by restriction digestion with XbaI and KpnI and ligation into pKNT25 cut with XbaI and KpnI.
pMB172	<i>ssgA</i> in pUT18 (Amp <sup>R</sup> )	<i>ssgA</i> was amplified with oligo <i>ssgA</i> -BACTH F/R followed by restriction digestion with XbaI and KpnI and ligation into pUT18 cut with XbaI and KpnI.
pMB173	<i>ssgB</i> in pKNT25 (Kan <sup>R</sup> )	<i>ssgB</i> was amplified with oligo <i>ssgB</i> -BACTH F/R followed by restriction digestion with BamHI and KpnI and ligation into pKNT25 cut with BamHI and KpnI.
pMB176	<i>ssgB</i> in pUT18 (Amp <sup>R</sup> )	<i>ssgB</i> was amplified with oligo <i>ssgB</i> -BACTH F/R followed by restriction digestion with BamHI and KpnI and ligation into pUT18 cut with BamHI and KpnI.

## References

1. Gregory MA, Till R, & Smith MC (2003) Integration site for *Streptomyces* phage FBT1 and development of site-specific integrating vectors. *J Bacteriol* 185(17):5320-5323.
2. Donczew M, *et al.* (2016) ParA and ParB coordinate chromosome segregation with cell elongation and division during *Streptomyces* sporulation. *Open Biol* 6(4):150263.
3. Paget MS, Chamberlin L, Atrih A, Foster SJ, & Buttner MJ (1999) Evidence that the extracytoplasmic function sigma factor  $\sigma^E$  is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). *J Bacteriol* 181(1):204-211.
4. Gust B, Challis GL, Fowler K, Kieser T, & Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci U S A* 100(4):1541-1546.
5. Fernández-Martínez LT & Bibb MJ (2014) Use of the meganuclease I-SceI of *Saccharomyces cerevisiae* to select for gene deletions in actinomycetes. *Sci Rep* 4:7100.
6. Hong HJ, Hutchings MI, Hill LM, & Buttner MJ (2005) The role of the novel Fem protein VanK in vancomycin resistance in *Streptomyces coelicolor*. *J Biol Chem* 280(13):13055-13061.
7. Karimova G, Pidoux J, Ullmann A, & Ladant D (1998) A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc Natl Acad Sci U S A* 95(10):5752-5756.