**Supplementary File 1**

**Table 1:** Bacterial strains used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Strain** | **Details** | **Source** | **Reference** |
| *S.venezuelae* | | | |
| NRRL B-65442 | Wild Type (WT) | Laboratory Strain | NZ\_CP018074.1 |
| DU669 | Δ*ftsZ::apr* |  | Santos-Beneit et al., 2017 |
| SS4 | WT *attBΦBT1*::pIIJ10257 | pIJ10257 integrated into the ΦBT1-attachment site of *S. venezuelae* | Schlimpert et al., 2017 |
| SS12 | WT *attB*ΦBT1::P*ftsZ-ftsZ-ypet* | pSS5 integrated into the ΦBT1-attachment site of *S. venezuelae* | Schlimpert et al., 2017 |
| SV56 | Δ*sepH*::*apr* | Chromosomal *sepH* locus was replaced by *apr-oriT* cassette amplified with primer mb118/mb119 and then transduced into WT using SV1 | This work |
| MB747 | Δ*sepH::apr* *attBΦBT1*::P*sepH-sepH* | pMB557 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB749 | Δ*sepH::apr* *attBΦBT1*::pIJ10770 | pIJ10770 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB750 | Δ*sepH::apr* *attBΦBT1*::P*ftsZ-ftsZ-ypet* | pSS5 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB751 | WT *attBΦBT1*::P*sepH-sepH-ypet attBΦC31*::P*ftsZ-ftsZ-mcherry* | pMB196 integrated into the ΦBT1-attachment site and pSS29 integrated into the ΦC31-attachment site of *S. venezuelae* | This work |
| MB763 | WT *attBΦBT1*::P*sepH-sepH-ypet* | pMB196 integrated into the ΦBT1-attachment site of *S. venezuelae* | This work |
| MB807 | WT *attBΦBT1*::P*sepH-sepH-ypet; PhupA-hupA-mcherry* | pMB583 integrated into the ΦBT1-attachment site of *S. venezuelae* | This work |
| MB827 | Δ*sepH::apr* *attBΦBT1*::P*sepH-sepH-CTD-y*pet | pMB592 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB828 | Δ*sepH::apr* *attBΦBT1*::P*sepH-sepH-NTD-y*pet | pMB591 integrated into the ΦBT1-attach-ment site of SV56 | This work |
| **Strain** | **Details** | **Source** | **Reference** |
| MB852 | Δ*sepH::apr* *attBΦBT1*::*PermE\*- sepH-NTD-ypet* | pMB621 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB851 | Δ*sepH::apr* *attBΦBT1*::*Z- sepH-CTD-ypet* | pMB619 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB856 | WT *attBΦBT1*::*PermE\*- sepH-NTD-ypet* | pMB621 integrated into the ΦBT1-attachment site of WT | This work |
| MB858 | WT *attBΦBT1*::*PermE*\*- *sepH-ypet* | pMB618 integrated into the ΦBT1-attachment site of *S. venezuelae* | This work |
| MB859 | Δ*ftsZ::apr* *attBΦBT1*::*PermE*\*- *sepH-ypet* | pMB618 integrated into the ΦBT1-attachment site of DU669 | This work |
| MB918 | Δ*sepH::apr* *attBΦBT1*::P*sepH-sepH-ypet* | pMB196 integrated into the ΦBT1-attachment site of SV56 | This work |
| MB938 | Δ*sepH::apr* *attBΦBT1*:: *PsepH*-*-sepH-G79P* | pMB688 integrated into the ΦBT1-attachment site of SV56 | This work |
| SS380 | Δ*sepH*::*apr* *attBBT1*::*PsepH*-*MSMEG*\_5685-*mcherry* | pSS491 integrated into the ΦBT1-attachment site of SV56 | This work |
| *E. coli* | | | |
| ET12567(pUZ8002) | *F– dam13::Tn9 dcm6 hsdM hsdR recF143::Tn10 galK2 galT22 ara-14 lacY1 xyl-5leuB6 thi-1 tonA31 rpsL hisG4 tsx-78 mtl-1 glnV44* | ET12567 containing helper plasmid pUZ8002 | Paget et al., 1999 |
| BW25113 | Δ(*araD*-*araB*)*567* Δ *lacZ4787*(::*rrnB-4*) *lacIp-4000*(*lacIQ*), l*-rpoS369*(*Am*) *rph-1* Δ(*rhaD*-*rhaB*)*568 hsdR514* | BW25113 containing λ RED recombination plasmid | Datsenko and Wanner, 2000 |
| DH5α | F- *fhuA2* (*argF-lacZ*)U169 *phoA* *glnV44* 80 (*lacZ*)M15 *gyrA96*  *recA1* *relA1* *endA1* *thi-1* *hsdR17* | Cloning and membrane protein topology analysis | NEB |
| Rosetta (DE3) | F- *ompT hsdS*B(rB- mB-) *gal dcm* (DE3) pRARE (CamR) | Host strain for protein overexpression | Merck |
| **Strain** | **Details** | **Source** | **Reference** |
| *S. cerevisiae* | | | |
| Y2HGold | *MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, met–, gal80Δ, MEL1, URA3::GAL1UAS -GAL1TATA-lacZ* | Host strain for yeast-two hybrid analysis | Clontech |

**Table 2:** Plasmids used in this study.

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Construction/References** |
| pDONR207 | Gateway entry vector (GentR) | Life Technologies |
| pGADCg | Yeast two-hybrid cloning vector, C-terminal GAL4 AD fusion (AmpR) | Clontech |
| pGADT7 | Yeast two-hybrid cloning vector, N-terminal GAL4 AD fusion (AmpR) | Clontech |
| pGBKCg | Yeast two-hybrid cloning vector, C-terminal GAL4 DNA-BD fusion (KanR) | Clontech |
| pGBKT7 | Yeast two-hybrid cloning vector, N-terminal GAL4 DNA-BD fusion (KanR) | Clontech |
| pET-21b | Plasmid for overexpression of proteins with N-terminal His6 fusion | Novagen |
| pTB145 | Plasmid for overexpression of His6-Upl1 protease (AmpR) | Bendezú et al., 2009 |
| pTB146 | Plasmid for overexpression of proteins with N-terminal His6-SUMO fusion (AmpR) | Bendezú et al., 2009 |
| pIJ10257 | Plasmid cloning vector for the conjugal transfer of DNA (under control of the *PermE\**constitutive promoter) from *E. coli* to *Streptomyces spp.* Integrates specifically at the ΦBT1 attachment site (HygR) | Hong et al., 2005 |
| pIJ773 | pBluescript KS (+) containing the apramycin resistance gene *apr* and *oriT* of plasmid RP4, flanked by FRT sites (AprR). Used as template for the amplification of the *apr oriT* cassette for ‘Redirect’ PCR-targeting (AprR) | Gust et al., 2003 |
| pIJ790 | Modified λ RED recombination plasmid *[oriR101] [repA101*(ts*)] araBp-gam-bet-exo* (CamR) | Gust et al., 2003 |
| **Name** | **Description** | **Construction/References** |
| pMS82 | Plasmid cloning vector for the conjugal transfer of DNA from *E. coli* to *Streptomyces* spp. Integrates site specifically at the ΦBT1 attachment site (HygR) | Gregory et al., 2003 |
| pIJ10770 | Modified pIJ10750 lacking an intrinsic apramycin promoter upstream of the extended multiple cloning site (HygR) | Schlimpert et al., 2017 |
| pSS5 | pMS82 carrying *PftsZ-ftsZ* (HygR) | Schlimpert et al., 2017 |
| pSS172 | pIJ10750 carrying *mcherry* (HygR) | Schlimpert et al., 2017 |
| pSS238 | pIJ10257 carrying *sepF* (HygR) | Schlimpert et al., 2017 |
| pSS245 | pIJ10257 carrying *sepF2* (HygR) | Schlimpert et al., 2017 |
| pSS250 | pGADT7 carrying *sepF2* (AmpR) | Schlimpert et al., 2017 |
| pSS250 | pGBKT7 carrying *sepF2* (KanR) | Schlimpert et al., 2017 |
| pSS253 | pGBT9 carrying *ftsZ* (AmpR) | Schlimpert et al., 2017 |
| pSS254 | pGAD424 carrying *ftsZ* (AmpR) | Schlimpert et al., 2017 |
| pSS269 | pGADT7 carrying *dynA* (AmpR) | Schlimpert et al., 2017 |
| pSS270 | pGADT7 carrying *dynB* (AmpR) | Schlimpert et al., 2017 |
| pSS271 | pGADT7 carrying *sepF* (AmpR) | Schlimpert et al., 2017 |
| pSS272 | pGADT7 carrying *sepF3* (AmpR) | Schlimpert et al., 2017 |
| pSS273 | pGBKT7 carrying *dynB* (KanR) | Schlimpert et al., 2017 |
| pSS274 | pGBKT7 carrying *dynA* (KanR) | Schlimpert et al., 2017 |
| pSS275 | pGBKT7 carrying *sepF* (KanR) | Schlimpert et al., 2017 |
| pSS276 | pGBKT7 carrying *sepF3* (KanR) | Schlimpert et al., 2017 |
| pFRL10 | pGBKCg carrying *sepH* (KanR) | Resulting from LR Gateway recombination between pFRL6-C and pGBKCg |
| pFRL11 | pGADCg carrying *sepH* (AmpR) | Resulting from LR Gateway recombination between pFRL6-C and pGADCg |
| pFRL12-N | pDONR207 carrying *sepH-NTD* (GentR) | Amplification of *sepH*1-186 using primers fr8/fr12 cloned into pDONR207 using BP Gateway reaction |
| pFRL14-N | pDONR207 carrying *sepH-CTD* (GentR) | Amplification of *sepH*187-344 using primers fr16/fr9 cloned into pDONR207 using BP Gateway reaction |
| pFRL15 | pGADT7 carrying *sepH-NTD* (AmpR) | Resulting from LR Gateway recombination between pFRL12-N and pGADT7 |
| pFRL23 | pGADT7 carrying *sepH-CTD* (AmpR) | Resulting from LR Gateway recombination between pFRL14-N and pGADT7 |
| pFRL39 | pTB146 carrying *sepH* (AmpR) | Amplification of *sepH* using primers fr34/fr35; amplification of pTB146 using primers 1219/1220. Assembly of both fragments using Gibson (NEB) |
| **Name** | **Description** | **Construction/References** |
| pFRL40 | pTB146 carrying *sepH-NTD* (AmpR) | Amplification of *sepH*1-186 using primers fr36/fr37; amplification of pTB146 using primers 1219/1220. Assembly of both fragments using Gibson (NEB) |
| pFRL41 | pTB146 carrying *sepH-CTD* (AmpR) | Amplification of *sepH*187-344 using primers fr38/fr39; amplification of pTB146 using primers 1219/1220. Assembly of both fragments using Gibson (NEB) |
| pFRL6-C | pDONR207 carrying *sepH* lacking stop codon (GentR) | Amplification of *sepH* using primers fr8/fr10 cloned into pDONR207 using BP Gateway reaction |
| pFRL6-N | pDONR207 carrying *sepH* including stop codon (GentR) | Amplification of *sepH* using primers fr8/fr9 cloned into pDONR207 using BP Gateway reaction |
| pFRL8 | pGBKT7 carrying *sepH* (KanR) | Resulting from LR Gateway recombination between pFRL6-N and pGBKT7 |
| pFRL9 | pGADT7 carrying *sepH* (AmpR) | Resulting from LR Gateway recombination between pFRL6-N and pGADT7 |
| pJUK10 | pGBKT7 carrying *sepHms* (KanR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK7 and pGBKT7 |
| pJUK12 | pGADT7 carrying *sepHms* (AmpR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK7 and pGADT7 |
| pJUK15 | pGBKCgcarrying *sepFms* (KanR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK8 and pGBKCg |
| pJUK17 | pGADCgcarrying *sepFms* (AmpR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK8 and pGADCg |
| pJUK19 | pGBKCgcarrying *ftsZms* (KanR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK9 and pGBKCg |
| pJUK21 | pGADCgcarrying *ftsZms* (AmpR) | Recombinant plasmid resulting from LR Gateway reaction between pJUK9 and pGADCg |
| pJUK7 | pDONR207 carrying *sepHms* (GentR) | Amplification of *sepHms* using primers JS25/JS26 cloned into pDONR207 using BP Gateway reaction |
| pJUK8 | pDONR207 carrying *sepFms* (GentR) | Amplification of *sepFms* using primers JS27/JS28 cloned into pDONR207 using BP Gateway reaction |
| pJUK9 | pDONR207 carrying *ftsZms* (GentR) | Amplification of *ftsZms* using primers JS29/JS30 cloned into pDONR207 using BP Gateway reaction |
| **Name** | **Description** | **Construction/References** |
| pMB196 | pMS82 carrying *PsepH-sepH-ypet* (HygR) | Two-step amplification using mb177/mb398 and mb399/mb261 in the first step. Templates from first step amplified using mb177 and mb261 to generate *PsepH-sepH-[SGGGG]*-*ypet* fragment, cloned via HindIII/KpnI into pMS82 |
| pMB557 | pIJ10770 carrying *PseH-sepH* (HygR) | Amplification of *PsepH-sepH* using mb177/mb213 and cloned into pIJ10770 via HindIII/KpnI |
| pMB583 | pSS305 carrying *PsepH-sepH-ypet* (HygR) | *psepH-sepH-ypet* was amplified by mb1019/mb1020 and cloned via EcoRV into pSS305 |
| pMB587 | pMS82 carrying *PsepH-sepH-CTD-y*pet (HygR) | Two-step amplification using pMB196 as a template and primer mb1032/mb1036 and mb1037/mb1035 in the first step. Templates from the first step were annealed and amplified using mb1032 and mb1035 to generate the *PsepH-sepH-CTD-y*pet fragment, cloned via HindIII/KpnI into pMS82 |
| pMB588 | pMS82 carrying *PsepH-sepH-NTD-ypet* (HygR) | Two-step amplification using pMB196 as a template and primer mb1032/mb1033 and mb1034/1035 in the first step. Templates from the first step were annealed and amplified using mb1032 and mb1035 to generate *PsepH-sepH-NTD-ypet* fragment, cloned via HindIII/KpnI into pMS82 |
| pMB591 | pIJ10770 carrying *PsepH-sepH-NTD-ypet* (HygR) | *PsepH-sepH-NTD-ypet* was sub-cloned from pMB588 via HindIII/KpnI |
| pMB592 | pIJ10770 carrying *PsepH-sepH-CTD-ypet* (HygR) | *PsepH-sepH-CTD-ypet* wassubcloned from pMB587 via HindIII/KpnI |
| pMB618 | pIJ10257 carrying *PsepH-sepH-ypet* (HygR) | Amplification of *sepH-ypet* using pMB196 as a template and primer mb1068/mb1069 and cloned into pIJ10257 using NdeI/HindIII |
| pMB619 | pIJ10257 carrying *sepH-CTD-ypet* (HygR) | Amplification of *PsepH-sepH-CTD-ypet* using pMB587 as a template and primer mb1070/mb1069 – cloned via NdeI/HindIII into pIJ10257 |
| pMB621 | pIJ10257 carrying *sepH-NTD-ypet* (HygR) | Amplification of *PsepH-sepH-NTD-ypet* using pMB588 as a template and primer mb1068/mb1069 – cloned via NdeI/HindIII into pIJ10257 |
| pMB674 | pGADT7 carrying *sepH-G79P* (AmpR) | *Pfu-UltraII*-Quikchange of pFRL9 using mb1122/mb1123 |
| pMB688 | pIJ10770 carrying *psepH-sepH-G79P* (HygR) | *Pfu-UltraII*-Quikchange of pMB557 using mb1122/mb1123 |
| **Name** | **Description** | **Construction/References** |
| pSS29 | pKF351 carrying *PftsZ-ftsZ-mcherry* | *ypet* was removed from pKF351 by restriction digestion with NdeI/BglII and replaced by *mcherry* |
| pSS287 | pTB146 carrying *ftsZ* (AmpR) | Insert and recipient vector were PCR amplified with oligo 452/453 and 451/454, respectively, followed by Gibson assembly |
| pSS305 | pSS172 carrying *hupA* (HygR) | Insert was PCR amplified with oligo ss103/ss104, cut with HindIII/AvrII followed by ligation into pSS172 cut with HindIII/AvrII |
| pSS472 | pIJ10257 carrying *msmeg\_5685-mcherry* (HygR) | Codon-optimised *msmeg\_5685* was PCR amplified with oligo ss1018/ss1020 and partially cut with NdeI/XhoI followed by ligation into pSS426 cut with NdeI-XhoI |
| pSS491 | pIJ10257-derivative carrying *msmeg\_5685-mcherry* (HygR) | *sepH* promoter region was PCR amplified with oligo ss1055/ss1056, cut with KpnI/NdeI. This fragment was used to replace the *PermE*\* fragment in pSS472 |
| pSS560 | pTB146 carrying *ftsZMs*(CarbR) | Amplification of *MSMEG\_4222* using oligo ss1221/ss1222 and assembled with pTB146 which was amplified with primer ss1219/ss1220 |
| pSS561 | pET21b carrying *sepHMs* (CarbR) | Amplification of *MSMEG\_5685* using oligo ss1225/ss1226 and insertion between the NdeI/HindIII site of pET21b via restriction cloning |

**Table 3: Oligonucleotides used in this work.**

|  |  |
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| **Primer** | **Sequence** |
| ss103 | attaAAGCTTgccgctcggacttcccgaaccag |
| ss104 | ttaattCCTAGGcttgcccttggcggcttccttgag |
| ss451 | ACCACCAATCTGTTCTCTGTGAG |
| ss452 | GAGAACAGATTGGTGGTATGGCAGCACCGCAGAAC |
| ss453 | GGAGCTCTGCTCTTCTTCACTTCAGGAAGTCCGGG |
| ss454 | AGAAGAGCAGAGCTCCGTCGAC |
| ss1018 | AATTAATTCATATGCGGGAGCTGCGCGTGGTGG |
| ss1020 | ATATACTCGAGCCGCTGGGTGCCCGACGACC |
| ss1055 | ATCGGGGTACCCCCGCTACCTGCACATCGACGG |
| ss1056 | AATTAATTCATATGTCACACGGTAGAGGGGTTCGCG |
| ss1219 | CTCCGTCGACAAGCTTGCGG |
| ss1220 | ACCACCAATCTGTTCTCTGTGAGCC |
| ss1221 | GAACAGATTGGTGGTATGACCCCCCCGCATAACTACCTCG |
| ss1222 | CCGGGCTCGAGTGCGTCAGTGCCGCATGAAGGGCGG |
| ss1225 | attaattcatatgcgagaactcagggtcgtcggac |
| ss1226 | attaAAGCTTgcgctgcgtacccgacgagc |
| fr10 | GGGGACCACTTTGTACAAGAAAGCTGGGTGGTCCTGCTTCTTCCGCCGCGTG |
| **Primer** | **Sequence** |
| fr12 | GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAGATCAGGGCCCTGGCCTC |
| fr16 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGCGAGACGGACGACACG |
| fr34 | ACAGAGAACAGATTGTGTGTATGCCCGAACTGCGTGTC |
| fr35 | CCGCAAGCTTGTCGACGGAGTCAGTCCTGCTTCTTCCG |
| fr36 | ACAGAGAACAGATTGGTGGTATGCCCGAACTGCGTGTC |
| fr37 | CCGCAAGCTTGTCGACGGAGTCAGCCGATCAGGGCCCT |
| fr38 | ACAGAGAACAGATTGGTGGTGGCGAGACGGACGACACG |
| fr39 | CCGCAAGCTTGTCGACGGAGTCAGTCCTGCTTCTTCCGC |
| fr8 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCCGAACTGCGTGTCGTGG |
| fr9 | GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAGTCCTGCTTCTTCCGCCG |
| JS25 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCGAGAACTCAGGGTCGTCG |
| JS26 | GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAGCGCTGCGTACCCGA |
| JS27 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGCACACTGCATAAGGT |
| JS28 | GGGGACCACTTTGTACAAGAAAGCTGGGTGACGGTAGGAGTAGAAGCCCG |
| JS29 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACCCCCCCGCATAACT |
| JS30 | GGGGACCACTTTGTACAAGAAAGCTGGGTGGTGCCGCATGAAGGGCGG |
| mb118 | CACGTGACGTCGGCAGGCACCACCCGGGAGGTCCCCATGATTCCGGGGATCCGTCGACC |
| mb119 | AGCCGCGGAACCGGCGGACCGCCACGGCTCCTGCCGTCATGTAGGCTGGAGCTGCTTC |
| mb144 | GGCAGGCCACACTCGACT |
| mb145 | ACCGGACCCGCAGTAGTC |
| mb177 | GGCGAAGCTTCCCCTGCTCCAGTCCCTG |
| mb213 | GGGGTACCGACCGCCACGGCTCCTG |
| mb261 | GGGGTACCTCACTTGTACAGCTCGTTCATG |
| mb398 | CTTGGAGACGCCGCCGCCGCCACTGTCCTGCTTCTTCCGCCG |
| mb399 | AAGCAGGACAGTGGCGGCGGCGGCGTCTCCAAGGGCGAGGAGC |
| mb1019 | GGGCGGATATCCCCCTGCTCCAGTCCCTG |
| mb1020 | GGGCGGATATCTCACTTGTACAGCTCGTTCATGC |
| mb1032 | GGCGAAGCTTCCCCTGCTCCAGTCCCTG |
| mb1033 | GCCGCCACTGATCAGGGCCCTGGCCTC |
| mb1034 | GCCCTGATCAGTGGCGGCGGCGGCGTC |
| mb1035 | GGGGTACCTCACTTGTACAGCTCGTTCATGCCC |
| mb1036 | CGTCTCGCCCATGGGGACCTCCCGGGT |
| mb1037 | GTCCCCATGGGCGAGACGGACGACACG |
| mb1068 | GGGAATTCCATATGCCCGAACTGCGTGTCGTGG |
| mb1069 | CCCAAGCTTTCACTTGTACAGCTCGTTCATGCCC |
| mb1070 | GGGAATTCCATATGGGCGAGACGGACGACACGA |
| mb1122 | GCCCAGATGGCCCCGATCCCCGTCGAC |
| mb1123 | GTCGACGGGGATCGGGGCCATCTGGGC |
| mb1124 | GGCATCGCCGAGCTCCGCG |
| mb1125 | GCGGTCGTCGCCGCGGGC |
| mb1126 | ATGAGCCATATTCAACGGGAAACGTC |
| mb1127 | CCATGCATCATCAGGAGTACGGATA |
| mb1129 | CCTTCGATCGTCCCTGGTCGGAGC |
| **Primer** | **Sequence** |
| mb1133 | GGCCTGGCGCTGGGGCTT |
| mb1136 | GCCCTTCGCGTCACGGCG |
| mb1138 | GCTGCTCGTCCCCGCGCA |
| mb1139 | GCGGCTGACACCGAGTGC |
| mb1140 | GATCATCGCGAGCCGCGGCAA |

**Supplementary References**

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