**Supplementary file 2: Plasmids**

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| **Plasmid name** | **Construct details** | | **Antibiotic marker** |  |
|  | |  |  |  |
| pNPTS138 | | (Skerker et al., 2005) | Kanamycin |  |
| pMCS1 | | (Thanbichler et al., 2007) | Spectinomycin |  |
| pYFPC1 | | (Thanbichler et al., 2007) | Spectinomycin |  |
| pXGFPC1 | | (Thanbichler et al., 2007) | Spectinomycin |  |
| pXYFPC1 | | (Thanbichler et al., 2007) | Spectinomycin |
| pXYFPC2 | | (Thanbichler et al., 2007) | Kanamycin |
| pNABC132 | | 600 bp internal fragment of *dnaE2* was amplified using AMJ\_oligo\_006 and AMJ\_oligo\_007 (reverse primer harboring mutations). Another 600 bp internal fragment of *dnaE2* was amplified using AMJ\_oligo\_008 (forward primer harboring mutations) and AMJ\_oligo\_009. These two fragments were assembled with linearized pNPTS138 vector using Gibson assembly. | Kanamycin |  |
| pNABC148 | | 600 bp fragments upstream and downstream of *dnaE2* genomic locus were amplified from *C. crescentus* gDNA using RR\_oligo\_021/RR\_oligo\_022 (upstream fragment) and RR\_oligo\_023/RR\_oligo\_024 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly. | Kanamycin |  |
| pNABC188 | | Full length *holB* was amplified from *C. crescentus* gDNA using AMJ\_038 and AMJ\_039, restriction digested with Nde1/Kpn1 and ligated to Nde1/Kpn1 digested pYFPC1 vector. *holB* and *YFP* in this construct was separated by 60 bp resulting in a 20 amino acid linker in the fusion protein. | Spectinomycin |  |
| pNABC198 | | Full length *dnaN* was amplified using AB\_036 and AB\_039, digested with Nde1/EcoR1 and ligated to Nde1/EcoR1 digested pYFPC1 vector. *dnaN* and *YFP* in this construct was separated by 36 bp resulting in a 12 amino acid linker in the fusion protein. | Spectinomycin |  |
| pNABC199 | | C-terminal region of *dnaE* was amplified using AB\_oligo\_791 and RR\_oligo\_004, restriction digested with NdeI/KpnI and ligated to NdeI/KpnI digested pYFPC1 plasmid. This region was further amplified from this construct using forward primer (AMJ\_oligo\_16) with 5' homology for region upstream of Nde1 and reverse primer (AMJ\_oligo\_17) with 5' homology for the region downstream of Kpn1 site in pMCS1 vector. Similarly, mNeonGreen fragment was amplified using forward primer (AMJ\_oligo\_18) with 5' homology for region downstream of Kpn1 and reverse primer (AMJ\_oligo\_15) with 5' homology downstream of Nhe1 site in pMCS1 vector. These amplicons and Nde1/Nhe1 digested pMCS1 vector were assembled using Gibson assembly to generate a construct where *dnaE* C-terminal was cloned in frame with *mNeonGreen*, separated by 60 bp resulting in a 20 amino acid linker in the fusion protein. | Spectinomycin |  |
| pNABC273 | | C-terminal region of *dnaE2* was amplified using RR\_oligo\_003 and IS\_oligo\_047 (harboring 5' overhangs for 3X-flag sequence), digested with Nde1/Nhe1, and ligated to Nde1/Nhe1 digested pMCS1 vector. | Spectinomycin |  |
| pNABC415 | | *ssb* fragment from pNABC419 was retrieved by Nde1/Kpn1 digestion and ligated to Nde1/Kpn1 digested pXGFPC1 plasmid. | Spectinomycin |  |
| pNABC416 | | 600 bp fragments upstream and downstream of *mutL* genomic locus were amplified from *C. crescentus* gDNA using PS\_oligo\_049/AMJ\_oligo\_061 (upstream fragment) and AMJ\_oligo\_062/PS\_oligo\_054 (downstream fragment) primer pairs. These fragments were assembled with linearized pNPTS138 vector using Gibson assembly. | Kanamycin |  |
| pNABC417 | | 600 bp fragments upstream and downstream of *uvrA* genomic locus were amplified from *C. crescentus* gDNA using PS\_oligo\_037/AMJ\_oligo\_057 (upstream fragment) and AMJ\_oligo\_058/PS\_oligo\_042 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly. | Kanamycin |  |
| pNABC418 | | *dnaN-YFP* fragment from pNABC198 construct was retrieved by restriction digestion with Nde1/Nhe1 and ligated to Nde1/Nhe1 digested pXYFPC1 plasmid. | Spectinomycin |  |
| pNABC419 | | Full length *ssb* amplified was using AB\_oligo\_658 and AB\_oligo\_659, digested with Nde1/Kpn1 and ligated with Nde1/Kpn1 digested pXYFPC2. *ssb* and *YFP* in this construct was separated by 60 bp resulting in a 20 amino acid linker in the fusion protein. | Kanamycin |  |
| pNABC420  pNABC438 | | pXYFPC2 vector was amplified using AB\_oligo\_651 and AB\_oligo\_652 and P*sidA*-*YFP* fragment was amplified from a replicating plasmid habouring YFP under P*sidA* promoter using AC\_oligo\_322 and AC\_oligo\_321. The vector and insert fragments were assembled with Gibson assembly.  600 bp fragments upstream and downstream of *imuB* genomic locus were amplified from *C. crescentus* gDNA using RR\_oligo\_017/RR\_oligo\_018 (upstream fragment) and RR\_oligo\_019/RR\_oligo\_020 (downstream fragment) primer pairs. These fragments were assembled with linearized pNPTS138 vector using Gibson assembly. | Kanamycin  Kanamycin |  |

**References**

Skerker, J. M., Prasol, M. S., Perchuk, B. S., Biondi, E. G., & Laub, M. T. (2005). Two-Component Signal Transduction Pathways Regulating Growth and Cell Cycle Progression in a Bacterium: A System-Level Analysis. *PLoS Biology*, *3*(10), e334. https://doi.org/10.1371/journal.pbio.0030334

Thanbichler, M., Iniesta, A. A., & Shapiro, L. (2007). A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in Caulobacter crescentus. *Nucleic Acids Research*, *35*(20), e137. https://doi.org/10.1093/nar/gkm818