**SUPPLEMENTARY FILE 1. PLASMIDS, DNA, AND PROTEIN SEQUENCES**

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| **Plasmids/DNA** | **Description** | **Source** |
| pET21b::*Caulobacter* ParB-His6 | Overexpression of C-terminally His6-tagged *Caulobacter* ParB, carbenicillinR>*Caulobacter* ParB-His6MSEGRRGLGRGLSALLGEVDAAPA**Q35**APGEQLGGSREAPIEILQRNPDQPRRTFREEDLEDLSNSIREKGVLQPILVRPSPDTAGEYQIVAGER**R104**WRAAQRAGLKTVPIMVRELDDLAVLEIGIIENVQRADLNVLEEALSYKVLMEKFERTQENIAQTIGKSRSHVANTMRLLALPDEVQSYLVSGELTAGHARAIAAAADPVALAKQIIEGGLSVRETEALARKAPNLSAGKSKGGRPPRVKDTDTQALESDLSSVLGLDVSIDHRGSTGTLTITYATLEQLDDL**C297**NRLTRGIKLAAALEHHHHHH\* (numbering according to 1) | Gift from C. Jacob-Wagner 2 |
| pET21b::*Caulobacter* ParB-His6 (C297S) | Overexpression of C-terminally His6-tagged *Caulobacter* ParB (C297S), carbenicillinR | This study |
| pET21b::*Caulobacter* ParB-His6 (Q35C C297S) | Overexpression of C-terminally His6-tagged *Caulobacter* ParB (Q35C C297S), carbenicillinR | This study |
| pET21b::TetR-His6 | Overexpression of C-terminally His6-tagged TetR (class B, from Tn10), carbenicillinR>TetR (class B, from Tn10)-His6MSRLDKSKVINSALELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALLDALAIEMLDRHHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGAKVHLGTRPTEKQYETLENQLAFLCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQAIELFDHQGAEPAFLFGLELIICGLEKQLKCESGSKLAAALEHHHHHH\* | This study |
|  pUC19::260bp-*parS*  | pUC19 plasmid with 260-bp insert that contains *parS* sites, carbenicillinR>260-bp\_natural\_*Caulobacter*\_*parS*\_fragment\_cloned\_into\_pUC19caagacgctcgcctcaatgcgaacgcccccgggttcgagcgggggcgctggactcgatctatacgccaatcaggcgagcgggtcgatgtgactcatcggcgtttcacgtgaaacacccccaccgcagctgtgagcggcctgtggacaatattggggatgttccacgtgaaacatcacttgccgatacagaaggtcgaaaagacccgtccaagaacgtcctcaggatcgatacggccggagatgcgctccagggcccgggc | This study |
| pUC19::260bp-scrambled *parS* | pUC19 plasmid with 260-bp insert that contains scrambled *parS* sites, carbenicillinR>260-bp\_scrambled\_*Caulobacter*\_*parS*\_fragment\_cloned\_into\_pUC19caagacgctcgcctcaatgcgaacgcccccgggttcgagcgggggcgctggactcgatctatacgccaatcaggcgagcgggtcgatgtgactcatcggacagctcgagattcatcccccaccgcagctgtgagcggcctgtggacaatattggggaatcgagtatacgctactcacttgccgatacagaaggtcgaaaagacccgtccaagaacgtcctcaggatcgatacggccggagatgcgctccagggcccgggc | This study |
| pET-His-MBP-TEV-DEST::*Sinorhizobium meliloti* ParB | For the purification of *Sinorhizobium meliloti* His6-MBP-ParB | 3 |
| pET-His-MBP-TEV-DEST::*Rhodobacter sphaeroides* ParB | For the purification of *Rhodobacter sphaeroides* His6-MBP-ParB | This study |
| pET-His-MBP-TEV-DEST::*Thermus thermophilus* ParB | For the purification of *Thermus thermophilus* His6-MBP-ParB | 3 |
| pET-His-MBP-TEV-DEST::*Dechloromonas aromatica* ParB | For the purification of *Dechloromonas aromatica* His6-MBP-ParB | This study |
| pET-His-MBP-TEV-DEST::*Psychrobacter* spp. ParB | For the purification of *Psychrobacter* spp. His6-MBP-ParB | This study |
| pET-His-MBP-TEV-DEST::*Staphylococcus aureus* ParB | For the purification of *Staphylococcus aureus* His6-MBP-ParB | 3 |
| pET-His-MBP-TEV-DEST::*Zymomonas mobilis* ParB | For the purification of *Zymomonas mobilis* His6-MBP-ParB | This study |
| pET-His-MBP-TEV-DEST::*Xanthomonas campestris* ParB | For the purification of *Xanthomonas campestris* His6-MBP-ParB | 3 |
| 169bp\_*parS* | cgccagggttttcccagtcacgacgttgtaaaacgacggccagtgaattcgagctcggtacccgcaggaggacgtagggtaggggga**tgtttcacgtgaaaca**ggggatcctctagagtcgacctgcaggcatgcaagcttggcgtaatcatggtcatagctgtttcct | This study |
| 169bp\_scrambled\_*parS* | cgccagggttttcccagtcacgacgttgtaaaacgacggccagtgaattcgagctcggtacccgcaggaggacgtagggtaggggga***aattacactgagttta***ggggatcctctagagtcgacctgcaggcatgcaagcttggcgtaatcatggtcatagctgtttcct | This study |
| 170bp\_*parS* | cgccagggttttcccagtcacgacgttgtaaaacgacggccagaattcgcaacgtg**tgtttcacgtgaaaca**gccttgaactgataacgactctatcattgatagagtgttctctccacgggatccccaggcatgcaagcttggcgtaatcatggtcatagctgtttcct | This study |
|  around\_pUC19\_F | tcactcatggttatggcagcactgcataattc | This study |
|  around\_pUC19\_F | taacactgcggccaacttacttctgacaacg | This study |
| 20bp\_*parS*\_BLI\_probeF | [Biotin]GGGAtgTTTCACGTGAAAca | This study |
| 20bp\_*parS*\_BLI\_probeR | tgTTTCACGTGAAAcaTCCC | This study |
| 28bp\_*tetO*\_BLI\_probeF | [Biotin]ggggactctatcattgatagagtatgc | This study |
| 28bp\_*tetO*\_BLI\_probeR | gcatactctatcaatgatagagtcccc | This study |
| 20bp\_*NBS*\_BLI\_probeF | [Biotin]GGGAtaTTTCCCGGGAAAta | This study |
| 20bp\_*NBS*\_BLI\_probeR | taTTTCCCGGGAAAtaTCCC | This study |

**Keys:**

M13F (-47): cgccagggttttcccagtcacgac M13R: aggaaacagctatgaccat

*parS:* **tgtttcacgtgaaaca** scrambled *parS*: ***aattacactgagttta***

*tetO:* actctatcattgatagagt *Bam*HI RS: ggatcc *Eco*RI RS: gaattc

**SUPPLEMENTARY REFERENCES**

1. Tran, N. T. *et al.* Permissive zones for the centromere-binding protein ParB on the Caulobacter crescentus chromosome. *Nucleic Acids Res* **46**, 1196–1209 (2018).

2. Lim, H. C. *et al.* Evidence for a DNA-relay mechanism in ParABS-mediated chromosome segregation. *Elife* **3**, e02758 (2014).

3. Jalal, A. S. B. *et al.* Evolving a new protein-DNA interface via sequential introduction of permissive and specificity-switching mutations. *bioRxiv* 724823 (2019) doi:10.1101/724823.