Supplementary Material

The *Bacillus subtilis* conjugative plasmid pLS20 encodes two ribbon-helix-helix type auxiliary relaxosome proteins that are essential for conjugation

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**Supplemental Table 1**

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| **Supplemental TableS1. Strains used** |
| **Strains** | **genotype or description** | **Source or Reference** |
| *E. coli* |  |  |
| XL1-Blue | *end*A1 *gyr*A96(nalR) *thi*-1 *rec*A1 *rel*A1 *lac* *gln*V44 F'[ ::Tn10 *pro*AB+ *lac*Iq Δ(*lac*Z)M15] *hsd*R17 (rK- mK+) | (Bullock et al., 1987) |
| BL21(DE3) | F– *ompT* *gal* *dcm* *lon* *hsdSB*(*rB*–*mB*–) λ(DE3 [*lacI* *lacUV5*-*T7 gene 1* *ind1* *sam7* *nin5*]) [*malB*+]K-12(λS) | laboratory stock |
| AZ37 | XL1-Blue strain harboring pET28b+ derivative pAND83 (containing *relLS20His(6)*) | (Ramachandran et al., 2017) |
| AZ42 | BL21(DE3) strain harboring pET28b+ derivative pAND83 (containing *relLS20His(6)*) | (Ramachandran et al., 2017) |
| BA50 | XL1-Blue strain harboring pET28b+ derivative pHJA56 (containing *aux1LS20His(6)*) | This work |
| BA54 | BL21(DE3) strain harboring pET28b+ derivative pHJA56 (containing *aux1LS20His(6)*) | This work |
| BA52 | XL1-Blue strain harboring pET28b+ derivative pHJA57 (containing *aux2LS20His(6)*) | This work |
| BA55 | BL21(DE3) strain harboring pET28b+ derivative pHJA56 (containing *aux2LS20His(6)*) | This work |
| *B. subtilis* |  |  |
| 168 (1A700) | *trpC2* | BGSC\* |
| PKS11 | 168 harboring pLS20cat | (Singh et al., 2012) |
| GR126 | *trpC2*, *amyE*::P*spank*-56-58 (Spec) | (Ramachandran et al., 2017) |
| GR127 | *trpC2,* *amy*E::P*spank*-*relLS20*, (Spec) | This work |
| GR149 | *trpC2,* pLS20Δ56-58 (Km, Cm) | (Ramachandran et al., 2017) |
| GR150 | *trpC2*, *amyE*::P*spank*-56-58 (Spec), pLS20Δ56-58 (Km, Cm) | (Ramachandran et al., 2017) |
| GR153 | *trpC2,* *amyE*::P*spank*-*relLS20* (Spec), pLS20Δ56-58 (Km, Cm) | This work |
| GR193 | *trpC2,* *amy*E::Pspank-*relLS20* (Spec), lacA::Pxyl-*56* (Em) | This work |
| GR197 | *trpC2,* *amy*E::Pspank-*57*-*58* (Spec), pLS20Δ56-58 (Km, Cm) | This work |
| GR200 | *trpC2,* *amy*E::Pspank-*relLS20*, *lac*A::Pxyl-*56* (Em), pLS20Δ56-58 (Km, Cm) | This work |
| GR206 | *trpC2,* *amy*E::Pspank-*56-57* (Spec), pLS20Δ56-58 (Km, Cm) | This work |
| \*, BGSC:  *Bacillus* Genetic Stock Center, Department of Bioch*em*istry, The Ohio State University, Columbus, OH, USA. (<http://www.bgsc.org/> |

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