

S3 TABLE: *Bacillus subtilis* strains used in this study

| Strains | Genotype | Source |
|---------|---|------------------------|
| 168 | <i>Prototrophic wild-type</i> | (Zeigler et al., 2008) |
| bAT87 | <i>amyE::PspollQ-cfp(Bs) (cat)</i> | This work |
| bBK3 | <i>ssdC::erm</i> | This work |
| bBK15 | <i>ywjI::PspollQ-cfp (cat)</i> | This work |
| bBK17 | <i>ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bBK18 | <i>ssdC::erm, ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bBK20 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm</i> | This work |
| bBK21 | <i>ycgO::PssdC-opt_{RBS}-SFgfp-ssdC (spec), ssdC::erm</i> | This work |
| bBK28 | <i>spoVID::kan</i> | This work |
| bBK33 | <i>safA::kan</i> | This work |
| bBK39 | <i>ylmD::kan, ssdC::erm</i> | This work |
| bBK41 | <i>yqhH::kan, ssdC::erm</i> | This work |
| bBK42 | <i>yqhG::kan, ssdC::erm</i> | This work |
| bBK43 | <i>spoVID::kan, ssdC::erm</i> | This work |
| bBK44 | <i>rpsT::kan, ssdC::erm</i> | This work |
| bBK45 | <i>yteV::kan, ssdC::erm</i> | This work |
| bBK46 | <i>skfA::kan, ssdC::erm</i> | This work |
| bBK47 | <i>ctpB::kan, ssdC::erm</i> | This work |
| bBK48 | <i>safA::kan, ssdC::erm</i> | This work |
| bBK49 | <i>cotZ::kan, ssdC::erm</i> | This work |
| bBK50 | <i>yqfT::kan, ssdC::erm</i> | This work |
| bBK51 | <i>mcsA::kan, ssdC::erm</i> | This work |
| bBK52 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spoIIAH::kan</i> | This work |
| bBK54 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spoVID::kan</i> | This work |
| bBK56 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, safA::kan</i> | This work |
| bBK57 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spoIID::kan</i> | This work |
| bBK60 | <i>ssdC::erm, spoVID::kan, ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bBK62 | <i>ssdC::erm, safA::kan, ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bBK64 | <i>spoVID::kan, ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bBK66 | <i>safA::kan, ywjI::PspollQ-cfp (cat), amyE::PspollD-yfp (spec)</i> | This work |
| bCR1565 | <i>ssdC::lox72</i> | This work |
| bHC25 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC(P174A) (spec), ssdC::erm</i> | This work |
| bHC27 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC(P238A) (spec), ssdC::erm</i> | This work |
| bHC29 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC(Y261A) (spec), ssdC::erm</i> | This work |
| bHC31 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC(F267A) (spec), ssdC::erm</i> | This work |
| bHC33 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC(E272A) (spec), ssdC::erm</i> | This work |
| bHC45 | <i>ycgO::PssdC-ssdC-His6 (spec), ssdC::erm</i> | This work |
| bHC70 | <i>spollQ::cat, ycgO::PssdC-RBS-ssdC-His6 (spec), ssdC::erm</i> | This work |
| bHC78 | <i>cotE::kan, ssdC::erm</i> | This work |
| bHC99 | <i>ssdC::erm, ycgO::PssdC-opt_{RBS}-CFP(8aa)-ssdC (spec), spoVD::markerless, spoVE::kan</i> | This work |
| bHC144 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (S118A) (spec), ssdC::erm</i> | This work |
| bJL3 | <i>ywjI::PspollQ-cfp (cat), spoVE::kan, spoVD::spec</i> | This work |
| bJL10 | <i>ssdC::erm, spoVIDΩspoVID-gfp (spec)</i> | This work |
| bJL12 | <i>spoVIDΩspoVID-gfp (spec)</i> | This work |
| bJL13 | <i>ycgO::PsafA-safA-mYPET (spec)</i> | This work |
| bJL33 | <i>spoVM::erm, ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::lox72</i> | This work |
| bJL34 | <i>spoIVA::cat, ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm</i> | This work |
| bJL35 | <i>ycgO::PsafA-safA-mYPET (spec), ssdC::erm</i> | This work |
| bJL39 | <i>spoVM::erm, ywjI::PspollQ-cfp (cat)</i> | This work |
| bJL40 | <i>spoIVA::cat, ycgO::PssdC-opt_{RBS}-sGFP(15aa)-ssdC (spec)</i> | This work |

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| bJL43 | <i>lacA::PspollQ-cfp(erm), spoIVA::cat</i> | This work |
| bJL44 | <i>spoVE::kan, spoVD::spec, ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm</i> | This work |
| bJL56 | <i>amyE::PspollQ-cfp(Bs) (cat), ssdC::erm</i> | This work |
| bJL59 | <i>spoIVA::cat</i> | This work |
| bJL66 | <i>yycR::PsspB-rbsopt-cfp (phleo), ssdC::erm</i> | This work |
| bJL78 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat</i> | This work |
| bJL79 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat, ssdC::erm</i> | This work |
| bJL80 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat, ssdC::erm, spoVID::kan</i> | This work |
| bJL81 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat, ssdC::erm, safA::kan</i> | This work |
| bJL82 | <i>ssdC::erm, ywjl::PspollQ-cfp (cat), spoVE::kan, spoVD::spec</i> | This work |
| bJL107 | <i>yoch::kan, ssdC::erm</i> | This work |
| bJL108 | <i>yodJ::kan, ssdC::erm</i> | This work |
| bJL109 | <i>yceE::kan, ssdC::erm</i> | This work |
| bJL110 | <i>yjgD::kan, ssdC::erm</i> | This work |
| bJL111 | <i>dltD::kan, ssdC::erm</i> | This work |
| bJL112 | <i>ypbE::kan, ssdC::erm</i> | This work |
| bJL114 | <i>yqhQ::kan, ssdC::erm</i> | This work |
| bJL115 | <i>yrrL::kan, ssdC::erm</i> | This work |
| bJL117 | <i>yxiS::kan, ssdC::erm</i> | This work |
| bJL118 | <i>ymzB::kan, ssdC::erm</i> | This work |
| bJL119 | <i>yrrl::kan, ssdC::erm</i> | This work |
| bJL120 | <i>yacL::kan, ssdC::erm</i> | This work |
| bJL121 | <i>dltB::kan, ssdC::erm</i> | This work |
| bJL129 | <i>ycgO::PspoIVA-mYPET-spoIVA (cat)</i> | This work |
| bJL133 | <i>spoVMΩspoVM-gfp (spec)</i> | This work |
| bJL135 | <i>spoVMΩspoVM-gfp (spec), ssdC::erm</i> | This work |
| bJL136 | <i>spoIVA::neo, ycgO::PspoIVA-mYPET-spoIVA (cat)</i> | This work |
| bJL140 | <i>ssdC::erm, ycgO::PspoIVA-mYPET-spoIVA (cat)</i> | This work |
| bJL158 | <i>spoVIDΩspoVID-gfp (spec), safA::kan</i> | This work |
| bJL159 | <i>ycgO::PsafA-safA-mYPET (spec), spoVID::kan</i> | This work |
| bJL160 | <i>ycgO::PsafA-safA-mYPET (spec), ssdC::erm, spoVID::kan</i> | This work |
| bJL162 | <i>ssdC::erm, spoVIDΩspoVID-gfp (spec), safA::kan</i> | This work |
| bJL175 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spollQ::cat</i> | This work |
| bJL176 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spollP::tet</i> | This work |
| bJL177 | <i>ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spollD::kan, spollP::tet</i> | This work |
| bJL178 | <i>yycR::PsspB-rbsopt-cfp (phleo), spollIAH (spec), ssdC::erm</i> | This work |
| bJL179 | <i>yycR::PsspB-rbsopt-cfp (phleo), spollIAH (spec), gerM::erm</i> | This work |
| bJL185 | <i>spollQ::erm, ycgO::PspoIVA-mYPET-spoIVA (cat)</i> | This work |
| bJL187 | <i>spollQ::erm, spoVMΩspoVM-gfp (spec)</i> | This work |
| bJL188 | <i>spoVMΩspoVM-gfp (spec), spollP::tet, spollD::kan</i> | This work |
| bJL189 | <i>ycgO::PspoIVA-mYPET-spoIVA (cat), spollP::tet, spollD::kan</i> | This work |
| bJL190 | <i>safA::tet, ycgO::PssdC-opt_{RBS}-cfp-ssdC (spec), ssdC::erm, spoVID::kan</i> | This work |
| bJL193 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat, spoVID::kan</i> | This work |
| bJL196 | <i>safA::tet, spoVID::kan, ywjl::PspollQ-cfp (cat), amyE::PspollD-yfp(JF)(spec)</i> | This work |
| bJL199 | <i>amyE::PspollQ-malF(2TM)-gfpΩcat, safA::kan</i> | This work |
| bKH21 | <i>yycR::PsspB-rbsopt-cfp (phleo)</i> | This work |
| bKH23 | <i>yycR::PsspB-rbsopt-cfp (phleo), spollIAH (spec)</i> | This work |