Supporting Information

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SI Text

Bacterial Strains, Phages, and Growth Conditions. *Escherichia coli* strain DH5 α (Table S1), used for cloning, was grown in LB medium. When appropriate, ampicillin was added to cultures and plates at a final concentration of 100 µg/mL. *Bacillus subtilis* strains were grown at 37 °C in LB medium containing 5 mM MgSO₄ and supplemented with appropriate antibiotics: kanamycin (5 µg/mL), erythromycin (1 µg/mL), and/or spectinomycin (100 µg/mL). *B. subtilis mreB* mutant strains (Table S1) can be propagated with near wild-type growth rate and cell morphology in growth medium supplemented with high concentrations of magnesium (1). Thus, when cytoskeleton mutants were used, MgSO₄ concentrations were increased to 25 mM in all cultures.

Generally, overnight cultures were diluted 1:50 in fresh medium and incubated for 2–3 h to reestablish exponential growth before manipulation. Expression of YFP fusions was induced by addition of 0.5% xylose at the time of phage infection. To express CFP fusions under a hyper-spank promoter, the culture media were supplemented with 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) at the time of infection.

Phage plaque assays were done by standard methods (2).

Plasmid Construction. The *yfp* fusion of ϕ 29 gene 3, encoding the terminal protein (TP), was constructed as follows. Gene 3 was amplified by PCR from \$\$\phi29 DNA using primer sets YFP-TP_U and YFP-TP_L (Table S3). The PCR product obtained was digested with XhoI/EcoRI and cloned into the B. subtilis amyE integration vector pSG5472 (3) digested with the same enzymes. As a result, gene 3 fused in frame to the yfpmut2 gene at its C terminus (pSGDM5) was located behind the xylose-inducible promoter P_{xvl} . pSGDM5 was used to transform competent B. subtilis cells. Spectinomycin-resistant transformants were tested for their ability to degrade starch to select for double-crossover transformants. To generate construct pDP150-TP, a PCR product containing gene 3 was amplified from the ϕ 29 genome by using primers TP_1 and TP_2. This fragment was digested with restriction enzymes NheI and SphI and cloned into equivalent sites of the thrC-integrating vector pDP150 (4), which contains the IPTG-inducible P_{hyper-spank} promoter. To generate plasmid pDP150-CFP, a PCR product containing the cfp(Bs) gene and a B. subtilis ribosome-entry site was amplified from plasmid pDR200 (5) by using primers CFP(BS)_U and CFP(BS)_L. This fragment was digested with restriction enzymes HindIII and NheI and cloned into equivalent sites of the thrC-integrating vector pDP150 (4). Plasmid pDP150-CFP-2 was generated by using the Quik-Change site directed-mutagenesis kit (Stratagene) and primers 150-SpeI-1 and 150-SpeI-2 to introduce a SpeI restriction site at the multiple cloning site (MCS) of the vector. To generate constructs pDP150-CFP/TP, pDP150-CFP/TP N-terminal (TP-Nt), pDP150-CFP/TP N-terminal/intermediate (TP-NtI), pDP150-CFP/ intermediate TP (TP-I), pDP150-CFP/intermediate/C-terminal TP (TP- Δ Nt), and pDP150-CFP/C-terminal TP (TP-Ct), specific regions of the $\phi 29$ gene 3 were amplified using the following primer sets: TP_U and TP_L, TP_U and TP-Nt_L, TP_U and TP-NtI_L, TP-I_U and TP-I_L, TP-I_U and TP_L and TP-Ct_U and TP_L, respectively. Each PCR product was digested with SphI and SpeI and cloned independently into the SphI and SpeI sites of plasmid pDP150-CFP-2. The plasmids obtained were used to transform competent B. subtilis cells, and erythromycin-resistant doublecrossover transformants were selected by the loss of spectinomycin tolerance. To construct plasmid pET-TP/NtI, the DNA sequence of ϕ 29 gene 3 spanning nucleotides 1–519 was amplified

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with primers TP-NcoI-U and TP-NtI-NotI-L. The PCR product obtained was digested with NcoI and NotI and cloned into the expression plasmid pET-28b(+) (Novagen) digested with the same enzymes. Likewise, plasmids pET-TP/Nt, pET-TP/\DeltaNt, and pET-TP/Ct were constructed using the following primer sets to amplify the desired regions of gene 3: TP-NdeI U and TP-Nt-NotI L, TP-I-NdeI U and TP-BamHI L, and TP-Ct-NdeI U and TP-BamHI L, respectively. Such a DNA fragment contains a 5' NdeI site and a stop codon followed by a NotI or a BamHI site at the 3' end, as indicated by the primer name. These DNA fragments were digested with the corresponding restriction enzymes and cloned into plasmid pET-28b(+) digested with the same enzymes. To generate construct pDP150-CFP/TP8-PRD1, the PRD1 gene VIII was amplified using primers TP8-R and TP8-L. The PCR product was digested with SphI and SpeI and cloned into the SphI and SpeI sites of plasmid pDP150-CFP-2.

Protein Treatment with Thrombin. Proteins containing histidine tags were digested with thrombin using the thrombin cleavage capture kit (Novagen). Complete cleavage of TP mutants was confirmed by Tris-Tricine-SDS electrophoresis. The biotinylated thrombin used to cleave these mutants then was removed from the sample by using the streptavidin-agarose column provided with the kit. Finally, the samples containing the purified proteins were dialyzed against 50 mM Tris-HCl (pH 7.5) containing 7 mM 2-mercaptoethanol, 1 mM EDTA, 200 mM NaCl, and 50% glycerol.

Immunofluorescence Microscopy. Blocking buffer contained 0.5% (wt/vol) casein (Sigma). Affinity-purified rat polyclonal antibodies against p3 were used at 1:1,000 dilution, and incubations were carried out for 1 h at room temperature. Polyclonal antibodies were centrifuged for 10 min at 14,000 × g at 4 °C before use to precipitate possible antibody aggregates. All samples were mounted for epifluorescence microscopy in multispot microscope slides (C.A. Hendley, Essex, Ltd) and supplemented with 0.2 μ g/mL DAPI or with 1.34 μ g/mL TO-PRO-3, when required.

Image Acquisition and Image Analysis. Imaging acquisition was performed as described (6) using a Sony CoolSnap HQ cooled charge-coupled device camera (Roper Scientific) attached to a Zeiss Axiovert 200M microscope. The digital images were acquired and analyzed with MetaMorph version 6 software. Images of fluorescent samples were deconvolved within MetaMorph and assembled in Adobe Photoshop version 7. Image manipulation was kept to a minimum. For general purposes, images were scaled and then saved as eight-bit images.

Analysis of Viral DNA by Gel Electrophoresis. Synthesis of viral DNA in vivo was analyzed as described (7). Basically, total intracellular DNA was isolated at different times after infection and analyzed in 0.6% agarose gels.

Real-Time PCR. The primer sets R-OUT-SUPER and R-25 were used to amplify regions of the genome of phage ϕ 29 (Table S3). The data obtained for samples were interpolated to standard curves constructed with known amounts of phage DNA. The results are expressed as nanograms of DNA per milliliter of culture.

Yeast Two-Hybrid Experiments. B. subtilis ORFs including the hbs, noc, smc, scpA, and scpB genes and ϕ 29 genes 2 and 3 were amplified by PCR from strain 168 and ϕ 29 genomic DNA, respectively. The DNA fragments were cloned into the pGBDU bait vector (Ura⁺) and the pGAD prey vector (Leu⁺), fused to the C terminus of the GAL4 DNA-binding domain (BD) and the GAL4 activation domain (AD), respectively. The haploid strain PJ69-4a of *Saccharomyces cerevisiae* was transformed by different combinations of bait vectors. Ura⁺ colonies were mated with haploid PJ69-4 α strains containing various prey vectors, and diploids (Ura⁺ Leu⁺) were selected on synthetic complete medium lack-

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ing leucine and uracil. The diploid cells were tested for expression of the interaction phenotypes (His^+ and Ade^+) by replica-plating the diploids onto selective plates lacking histidine or adenine, as described previously (8). Interaction phenotypes were scored after incubation for 7 d at 30 °C. Specific interactions were shown to be reproducible and not associated with self-activation.

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Fig. 51. Genetic and transcriptional map of the ϕ 29 genome and mechanism of in vitro ϕ 29 DNA replication. (A) Map of the ϕ 29 genome. The direction of transcription and length of the transcripts are indicated by arrows, and the positions of genes are indicated by numbers. TD1 corresponds to the bidirectional transcriptional terminator located between the convergently transcribed late and right-side early operons. Black circles represent the TP covalently linked to the 5' DNA ends. (*B*) Overview of the in vitro ϕ 29 DNA replication mechanism. Replication starts by recognition of the p6-nucleoprotein complexed origins of replication by a TP/DNA polymerase heterodimer. The DNA polymerase then catalyses the addition of the first deoxyAMP to the TP present in the heterodimer complex. After a transitional step, these two proteins dissociate, and the DNA polymerase continues processive elongation until replication of the nascent DNA strand is completed. Replication is coupled to strand displacement. The ϕ 29-encoded SSB protein p5 binds to the displaced ssDNA strands and is removed by the DNA polymerase during later stages of the replication process. Continuous polymerization results in the generation of two fully replicated ϕ 29 genomes. Circles, TP; triangles, DNA polymerase; ovals, replication initiator protein p6; diamonds, SSB protein p5; de novo synthesized DNA is shown as beads on a string. Adapted from ref. 1.

1. Muñoz-Espín D, et al. (2009) The actin-like MreB cytoskeleton organizes viral DNA replication in bacteria. Proc Natl Acad Sci USA 106:13347-13352.



sus2(513)

Fig. 52. ϕ 29 TP fluorescent fusions are functional in vivo. (*A* and *B*) Complementation experiments using a *sus*3(91) mutant phage and *B. subtilis* strains DM-022 (expressing YFP), DM-021 (expressing YFP-TP), and MO-101-P [suppressing the nonsense mutation of *sus*3(91) mutant phage]. (*A*) Agarose gel electrophoresis analysis illustrating the amount of viral DNA accumulated by the *B. subtilis* strain DM-021. Xylose-induced cells were infected with a *sus*3(91) mutant phage at a multiplicity of infection (MOI) of 5, and aliquots were harvested and processed at the indicated times postinfection. Location of the ϕ 29 genome and *B. subtilis* chromosomal DNA is indicated. (*B*) ϕ 29 intracellular accumulated DNA was quantified by real-time PCR after infection at an MOI of 5 at the indicated times postinfection. Phage DNA production was expressed as nanograms of viral DNA per milliliter of culture. The amount of intracellular phage ϕ 29 DNA accumulated was analyzed by agarose gel electrophoresis (C) and real-time PCR (*D*) after infection of the following *B. subtilis* strains with a *sus*3(91) mutant phage: DM-024 (expressing CFP), DM-025 (expressing CFP-TP), DM-023 (expressing CFP-TP and YFP-p2), and MO-101-P (suppressor strain). IPTG- and/or xylose-induced cells were infected at an MOI of 5, harvested at the indicated times after infection, and processed as described in *Materials and Methods*. Locations of the ϕ 29 genome and *B. subtilis* chromosomal DNA are indicated. The amounts of accumulated phage DNA (nanograms of viral DNA per milliliter of culture) are expressed in the graph as a function of time after infection. (*E*) Exponentially growing *B. subtilis* cells of strain DM-023 (expressing CFP-TP and YFP-p2) were infected with ϕ 29 mutant phage *sus*2(513) containing a suppressible stop codon in the DNA polymerase-encoding gene 2 (1). Next, samples were mixed with liquid top agar containing 0.5% xylose, spread on LB agar plates, and incubated overnight at 37 °C. Plaque formation similar to

1. Moreno F, Camacho A, Viñuela E, Salas M (1974) Suppressor-sensitive mutants and genetic map of Bacillus subtilis bacteriophage φ 29. Virology 62:1–16.



Fig. S3. The N-terminal domain of the TP is important for efficient ϕ 29 DNA replication. DM-024 (expressing CFP), DM-025 (expressing CFP-TP), DM-029 (expressing CFP-TP Δ Nt), and DM-032 (expressing wild-type TP) cells were grown at 37 °C in LB medium supplemented with 2% glucose to an OD₆₀₀ of 0.4. Cells then were infected with a *sus3*(91) mutant phage at an MOI of 1, and IPTG was added to a final concentration of 1 mM. At the indicated times (minutes), cell samples were harvested, processed, and subjected to SDS/PAGE and Western blotting (*A*) or agarose gel electrophoresis (*B*) (*SI Text*). (*A*) As an internal control, *B. subtilis* 168 cells were infected with ϕ 29 wild-type phage, and the production of TP was analyzed at the indicated times postinfection. Arrows indicate positioning of CFP-TP, CFP-TP Δ Nt, and TP bands. (*B*) Location of the ϕ 29 genome and *B. subtilis* chromosomal DNA.



Fig. S4. YFP-p2 colocalizes with the bacterial nucleoid at early infection times. YFP, DAPI staining, and merged images of *B. subtilis* cells expressing xyloseinduced YFP-p2 (strain DM-020). Cells were grown to midexponential phase in LB medium supplemented with 2% glucose at 37 °C, and at an OD₆₀₀ of 0.4 the culture was supplemented with 0.5% xylose. Subsequently, the culture was divided, and half the culture was infected with a *sus2*(513) mutant phage at an MOI of 5. Samples were harvested 10 min after xylose addition (or 10 min after infection) and analyzed by fluorescence microscopy techniques (*Materials and Methods* and *SI Text*). For clarity, YFP fluorescent signals and DAPI staining are false-colored red and green, respectively.



Fig. S5. *B. subtilis* RNA polymerase β' subunit and ϕ 29 TP colocalize at the bacterial nucleoid. Phase-contrast, CFP, YFP, and merged images of typical cells (*B. subtilis* strain DM-033) simultaneously expressing IPTG-induced CFP-TP and xylose-induced YFP-rpoC fusion proteins. Cultures were grown at 37 °C in LB medium supplemented with 2% glucose. At an OD₆₀₀ of 0.4, cultures were additionally supplemented with 0.5% xylose and 1 mM IPTG. Samples were harvested and processed for fluorescence microscopy 30 min after the addition of the inductors. For clarity, YFP and CFP fluorescent signals are false-colored red and green, respectively.

	Ą	AD-TP	AD-HBsu	AD-Noc	AD-SMC	AD-ScpA	AD-ScpB
BD	9		0	3	3	0	3
BD-DNApol	Ð	۲					0
BD-HBsu	Ð						3
BD-Noc	•			۲			0
BD-SMC	9 .						0
BD-ScpA		۲	۲	0	0	•	0

Fig. S6. ϕ 29 TP does not interact with nucleoid-associated proteins of *B. subtilis*. ϕ 29 TP protein as GAL4-AD fusion was tested for interaction with full-length HBsu, Noc, SMC, and ScpA as baits (GAL4-BD fusions). No evidence of specific interaction/s was obtained. Interactions of ϕ 29 TP-DNA polymerase (red square), Noc-Noc, SMC-ScpA, ScpA-ScpA, and ScpA-ScpB were obtained as internal controls. The BD-TP fusion was not functional in yeast and thus has not been included in this assay. Auto-interaction was tested by crossing preys and baits with the BD and AD expressed from empty vectors. The BD-ScpB fusion resulted in self-activation and has not been included. The yeast two-hybrid assay was performed in duplicate with independent yeast clones.

Table S1. Strains used

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$E. coliE. coliDH5 a\psi80dlacZΔM15, recA1, endA1, gr/AB, thi-1, hsdR17(rc-, me+),supE44, relA1, deoK, \Delta(lacZYA argF) U169, phoALaboratory stockXL1 bluerecA1, endA1, gr/A95, thi-1, hsdR17(rc-, me+), supE44, relA1, lac,[F, proAB, lacTZΔM15, rint(0ter)]Laboratory stockDM-049YL1 blue containing plasmid pDP150.CFPPpDP150.CFPP → XL1 blue (Amp)pDP150.CFPT PpDP150.CFPT → XL1 blue (Amp)DM-051XL1 blue containing plasmid pDP150.CFPTpDP150.CFPTB+RD1 → XL1 blue (Amp)10NAtrpC2 spoA3 su-Moreno et al. (1)110NAtrpC2 spoA3 su-Moreno et al. (1)110NAtrpC2 spoA3 su-Moreno et al. (2)SWV215trpC2 clamyE-Pary/fp.p3 spc)pSGDM4 - 168 (5p)DM-020trpC2 (lamyE-Pary/fp.p3 spc)pSGDM4 - 168 (5p)DM-022trpC2 (lamyE-Pary/fp.p3 spc)pSGDM4 - 168 (5p)DM-024trpC2 (lamyE-Pary/fp.p3 spc)pDF150.CFPTP - 168 (Em)DM-025trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Em)DM-026trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-027trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-028trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-029trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-024trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-025trpC2 (lathrC:Phoperapar-Cfp-p3 erm)pDF150.CFPTP + 168 (Erm)DM-032trpC2 (lathrC:Phoperapar-Cfp$	Strain	Relevant genotype	Construction, source, or reference		
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XL1 bluerecA1, endA1, gyrA96, thi-1, hstRit 71(r_c^- , m_c^-), supE44, relA1, lac, (F, proAB, lach*2AMIS:T110(ter))Laboratory stockBL21(DE3)F, ompT, hsdBa(r_c^- , m_c^-), dcm, gal, \LOE3)Laboratory stockDM-049XL1 blue containing plasmid pDP150-CFPDDP150-CFP - XL1 blue (Amp)DM-051XL1 blue containing plasmid pDP150-CFPTDDP150-CFPT-PR2+NL1 blue (Amp)DM-051XL1 blue containing plasmid pDP150-CFPTPDDP150-CFPTP3+RD1 - XL1 blue (Amp)B. subtileMoreno et al. (1)188trpC2: considered wild-type strainBacilus Genetic Stock CenterM0-101-Pthr "goDA" su'Mellado et al. (2)SWV215trpC2 filepolA::kan)XL and Strauch (3)DM-020trpC2 (lamy::P _{xy} -t/P _D 2 spc)pSGDM3 - 168 (5p)DM-021trpC2 (lamy::P _{xy} -t/P _D 2 spc)pSGDM4 - 168 (5p)DM-023trpC2 (lamy::P _{xy} -t/P _D 2 spc)pSGDM4 - 168 (5p)DM-024trpC2 (lamy::P _{xy} -t/P _D 2 spc)pSGDM3 - DP150-CFP - 168 (5m)DM-025trpC2 (lamy::P _{xy} -t/P _D 2 spc)pSGDM4 - 168 (5p)DM-026trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm)DM-026trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm)DM-027trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm)DM-028trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm)DM-029trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm)DM-020trpC2 (la(thr::P _{hiper-spank} -cfp-p3 erm)pD150-CFP/TP + 168 (Erm) <td></td> <td>supE44, relA1, deoR, Δ(lacZYA-argF) U169, phoA</td> <td>-</td>		supE44, relA1, deoR, Δ (lacZYA-argF) U169, phoA	-		
BL21(DE3) F_r omp. T_r hors $A_0(r_p, m_p)$, den, gal, ADE3)Laboratory stockDM-049X1 blue containing plasmid pDP150-CFP/TPpDP150-CFP/TP - XL1 blue (Amp)DM-051X1 blue containing plasmid pDP150-CFP/TPpDP150-CFP/TP - XL1 blue (Amp)DM-051X1 blue containing plasmid pDP150-CFP/TPpDP150-CFP/TP-ARD1Subbilitsmoreno et al. (1)110NAtrpC2 sp0A3 su ⁻ Moreno et al. (1)168trpC2, considered wild-type strainBaillus Genetic Stock CenterMO-101-Pth/ ⁻ sp0A ⁻ su ⁺ Moreno et al. (2)SWV215trpC2 flampEr-P _{gr7} /fb-p2 sp0p5GDM3 - 168 (Sp)DM-020trpC2 flampEr-P _{gr7} /fb-p2 sp0p5GDM3 - 168 (Sp)DM-021trpC2 flampEr-P _{gr7} /fb-p2 sp0 (flthC:Phiper-space-Cfp-p3 erm)p5GDM3 - 168 (Sp)DM-023trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP - 168 (Sp)DM-024trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Srm)DM-025trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-026trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-026trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-026trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-027trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-030trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-031trpC2 (flthc::Phiper-space-Cfp-p3 erm)pDP150-CFP/TP-Nt - 168 (Erm)DM-032t	XL1 blue	recA1, endA1, gyrA96, thi-1, hsdR17(r_{κ}^- , m_{κ}^+), supE44, relA1, lac, [F', proAB, lacl ^q Z Δ M15::Tn10(tet ^r)]	Laboratory stock		
DM-049XL1 blue containing plasmid pDP150-CFPpDP150-CFP \rightarrow XL1 blue (Amp)DM-050XL1 blue containing plasmid pDP150-CFP/TP-RD1pDP150-CFP/TP \rightarrow XL1 blue (Amp)BACTORDM-051XL1 blue containing plasmid pDP150-CFP/TP-RD1pDP150-CFP/TP \rightarrow XL1 blue (Amp)BACTORSubUlisMoreno et al. (1)Bacillus Genetic Stock CenterBacillus Genetic Stock CenterMO-101-Pthr "poodA" su"Mallado et al. (2)SWV215trpC2 pheA1 Ω (spo0A::kan)Xu and Strauch (3)DM-020trpC2 $I(amp:E-P_{gr}/tp-22 sp)$ pSGDMA \rightarrow 168 (Sp)DM-021trpC2 $I(amp:E-P_{gr}/tp-22 sp)$ pSGDMA \rightarrow 168 (Sp)DM-022trpC2 $I(amp:E-P_{gr}/tp-22 sp)$ pSGDMA \rightarrow 168 (Sp)DM-023trpC2 $I(amp:E-P_{gr}/tp-22 sp)$ pSGDMA \rightarrow 168 (Sp)DM-024trpC2 $I(amp:E-P_{gr}/tp-23 sen)$ pDP150-CFP/T \rightarrow 168 (Erm)DM-025trpC2 $I(thr::P_{hoper-spack}-Cfp-29 sen)$ pDP150-CFP/T \rightarrow 168 (Erm)DM-026trpC2 $I(thr::P_{hoper-spack}-Cfp-29 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-027trpC2 $I(thr::P_{hoper-spack}-Cfp-29 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-028trpC2 $I(thr::P_{hoper-spack}-Cfp-20 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-029trpC2 $I(thr::P_{hoper-spack}-Cfp-20 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-030trpC2 $I(thr::P_{hoper-spack}-Cfp-20 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-031trpC2 $I(thr::P_{hoper-spack}-Cfp-20 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)DM-032trpC2 $I(thr::P_{hoper-spack}-Cfp-20 sen)$ pDP150-CFP/TP \rightarrow 168 (Erm)	BL21(DE3)	F^- , ompT, hsdS _B (r _B -, m _B -), dcm, gal, λ (DE3)	Laboratory stock		
DM-050XL1 blue containing plasmid pDP150-CFPTPpDP150-CFPTP= XL1 blue (Amp)DM-051XL1 blue containing plasmid pDP150-CFPTP-RD1pDP150-CFPTP8-RD1 - XL1 blue (Amp)SubtilisMoreno et al. (1)110NAtrpC2 spo0A3 su ⁻ Moreno et al. (1)168trpC2, considered wild-type strainBaillus Genetic Stock CenterMO-101-Pth/ ⁻ spo0A su ⁺ Mellado et al. (2)SWV215trpC2 (lamp:E:P _{sy1} /tp-2 spc)pSGDM3 - 168 (sp)DM-020trpC2 (lamp:E:P _{sy1} /tp-p2 spc)pSGDM3 - 168 (sp)DM-021trpC2 (lamp:E:P _{sy1} /tp-p2 spc) (lthrC:P _{hiper-spank} -Cfp-p3 erm)pSGDM3 - 168 (sp)DM-023trpC2 (lamp:E:P _{sy1} /tp-p2 spc) (lthrC:P _{hiper-spank} -Cfp-p3 erm)pDF150-CFPT - 168 (sp)DM-024trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 erm)pDP150-CFPT - 168 (srm)DM-025trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 erm)pDP150-CFPT - 168 (Erm)DM-026trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 erm)pDP150-CFPTT - 168 (Erm)DM-027trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 erm)pDP150-CFPTT - 168 (Erm)DM-028trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)DM-029trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)DM-021trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)DM-025trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)DM-026trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)DM-037trpC2 (lthrC:P _{hiper-spank} -Cfp-p3 arm)pDP150-CFPTT - 168 (Erm)D	DM-049	XL1 blue containing plasmid pDP150-CFP	pDP150-CFP \rightarrow XL1 blue (Amp)		
DM-051XL1 blue containing plasmid pDP150-CFP/TP-PRD1pDP150-CFP/TP8-PRD1 \rightarrow XL1 blue (Amp)8. subtilis110NAtrpC2 spo0A3 su ⁻ Moreno et al. (1)168trpC2, considered wild-type strainBacillus Genetic Stock CenterM0-101-Pthr" spo0A ⁻ su ⁻ Mellado et al. (2)SWV215trpC2 pheA1 (spp0A ⁻ :kan)Xu and Strauch (3)DM-020trpC2 (lamyE::P _{xy17} /fp.p3 spc)pSGDM3 \rightarrow 168 (Sp)DM-021trpC2 (lamyE::P _{xy17} /fp.p3 spc)pSGDM3 \rightarrow 168 (Sp)DM-022trpC2 (lamyE::P _{xy17} /fp.p3 spc)pSGDM3 \rightarrow 168 (Sp)DM-023trpC2 (lamyE::P _{xy17} /fp.p3 sm)pDP150-CFP \rightarrow 168 (frm)DM-024trpC2 (lamyE::P _{xy17} /fp.p3 sm)pDP150-CFP/TP \rightarrow 168 (frm)DM-025trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-026trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-027trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-028trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-029trpC2 (lthrC::Phiper-spark: Cfp.p3 lt erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-031trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-031trpC2 (lthrC::Phiper-spark: Cfp.p3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-032trpC2 (lthrC::Phiper-spark: Cfp.P3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-033trpC2 (lthrC::Phiper-spark: Cfp.P3 erm)pDP150-CFP/TP \rightarrow 168 (frm)DM-034trpC2 (lthrC::Phiper-spark: Cfp.P3 erm)pDP150	DM-050	XL1 blue containing plasmid pDP150-CFP/TP	pDP150-CFP/TP \rightarrow XL1 blue (Amp)		
8. subtilisMoreno et al. (1)110NA $trpC2$ poolA3 su ⁻ Moreno et al. (1)188 $trpC2$, considered wild-type strainBacillus Genetic Stock CenterMO-101-P trr' spoOA ⁻ su ⁺ Mellado et al. (2)SWV215 $trpC2$ $\Omega(any E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-020 $trpC2$ $\Omega(any E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-021 $trpC2$ $\Omega(any: E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-022 $trpC2$ $\Omega(any: E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-023 $trpC2$ $\Omega(any: E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-024 $trpC2$ $\Omega(any: E:: P_{yr} yfp-p2 spc)$ pSGDM3 \rightarrow 168 (5p)DM-025 $trpC2$ $\Omega(any: E:: P_{yr} yfp-p2 spc)$ pDF150-CFP \rightarrow 168 (Erm)DM-026 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-p3 erm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-026 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-p3 erm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-027 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-p3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-028 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-p3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-032 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-P3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-032 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-P3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-032 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-P3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-032 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-P3 arm)$ pDF150-CFP/TP \rightarrow 168 (Erm)DM-033 $trpC2$ $\Omega(antrc: P_{hiper-spank} - Cfp-P3 arm)$ <t< td=""><td>DM-051</td><td>XL1 blue containing plasmid pDP150-CFP/TP-PRD1</td><td>pDP150-CFP/TP8-PRD1 \rightarrow XL1 blue (Amp)</td></t<>	DM-051	XL1 blue containing plasmid pDP150-CFP/TP-PRD1	pDP150-CFP/TP8-PRD1 \rightarrow XL1 blue (Amp)		
110NAtrpC2 spoA3 su ⁻ Moreno et al. (1)168trpC2, considered wild-type strainBacillus Genetic Stock CenterMO-101-Pthr ⁻ spoA ⁻ su ⁺ Mellado et al. (2)SWV215trpC2 (heary £: P ₀ , ryft-p2 spc)pSGDM3 - 168 (5p)DM-020trpC2 $\Omega(any £: P_{ny}, ryft-p3 spc)$ pSGDM4 - 168 (5p)DM-021trpC2 $\Omega(any £: P_{ny}, ryft-p3 spc)$ pSGDM3 - 168 (5p)DM-022trpC2 $\Omega(any £: P_{ny}, ryft-p3 spc)$ pSGDM3, DP150-CFP - 168 (5p)DM-023trpC2 $\Omega(any E: P_{ny}, ryft-p3 spc)$ pDF150-CFP - 168 (Fm)DM-024trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPT-Nt - 168 (Fm)DM-025trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPT-Nt - 168 (Fm)DM-026trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-027trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-028trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-029trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-030trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-031trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pDP150-CFPTP-Nt - 168 (Fm)DM-032trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pD150-CFPTP-Nt - 168 (Fm)DM-031trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pD150-CFPTP-Nt - 168 (Fm)DM-032trpC2 $\Omega(thrtC:: P_{hiper-spank} < ft-p3 th erm)$ pD150-CFPTP - 168 (Fm) <td< td=""><td>B. subtilis</td><td></td><td></td></td<>	B. subtilis				
168trpC2, considered wild-type strainBacillus Genetic Stock CenterMO-101-Pthr" spo0A su"Mellado et al. (2)MO-101-Pthr" spo0A su"Mellado et al. (2)SWV215trpC2 $Q(amy E: P_{gry} f/p-2 spc)$ pSGDM3 - 168 (Sp)DM-021trpC2 $Q(amy E: P_{gry} f/p-2 spc)$ pSGDM4 - 168 (Sp)DM-021trpC2 $Q(amy E: P_{gry} f/p-2 spc)$ pSGSM72 - 168 (Sp)DM-022trpC2 $Q(amy E: P_{gry} f/p-2 spc)$ pSGSM72 - 168 (Sp)DM-024trpC2 $Q(atrrb: E: P_{inper-spank} - Cfp-2 spc)$ pDF150-CFP - 168 (Erm)DM-025trpC2 $Q(ttrh C: P_{inper-spank} - Cfp-2 sem)$ pDF150-CFPT - 168 (Erm)DM-026trpC2 $Q(ttrh C: P_{inper-spank} - Cfp-2 sem)$ pDF150-CFPT + 168 (Erm)DM-027trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 sem)$ pDF150-CFPTP+Nt - 168 (Erm)DM-028trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 sem)$ pDF150-CFPTP-Nt + 168 (Erm)DM-029trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-029trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-031trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-032trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-033trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-034trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-035trpC2 $Q(ttrh C: P_inper-spank - Cfp-2 secm)$ pDF150-CFPTP-Nt + 168 (Erm)DM-036trpC2 $Q(ttrh C: P_inper$	110NA	trpC2 spo0A3 su ⁻	Moreno et al. (1)		
MO-101-Pthr spoA/su*Mellado et al. (2)SWV215trpC2 pheA1 24(spoA::kan)Xu and Strauch (3)DM-020trpC2 (lamy:::-p _{ay} -yfp-p3 spc)pSGDM3 → 168 (Sp)DM-021trpC2 (lamy:::-p _{ay} -yfp-p3 spc)pSGDM4 → 168 (Sp)DM-022trpC2 (lamy:::-p _{ay} -yfp-p3 spc)pSGDM4 → 168 (Sp)DM-023trpC2 (lamy:::-p _{ay} -yfp-p3 ern)pDS150-CFP → 168 (Ern)DM-024trpC2 (lamy:::-p _{ay} -yfp-p3 ern)pDP150-CFP → 168 (Erm)DM-025trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/T → 168 (Erm)DM-026trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/TP-Nt → 168 (Erm)DM-026trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/TP-Nt → 168 (Erm)DM-026trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/TP-Nt → 168 (Erm)DM-027trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/TP-Nt → 168 (Erm)DM-028trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pDP150-CFP/TP-Nt → 168 (Erm)DM-029trpC2 (lthrC::-P _{hiper-spank} -Cfp-p3 ern)pD150-CFP/TP-Nt → 168 (Erm)DM-030trpC2 (lthrC::P _{hiper-spank} -Cfp-p3 ern)pD150-CFP/TP-Nt → 168 (Erm)DM-031trpC2 (lthrC::P _{hiper-spank} -Cfp-p3 ern)pD150-CFP/TP → 168 (Erm)DM-032trpC2 (lthrC::P _{hiper-spank} -Cfp-P3 ern)pD150-CFP/TP → 168 (Erm)DM-033trpC2 (lthrC::P _{hiper-spank} -Cfp-P3 ern)pD150-CFP/TP → 168 (Erm)DM-034trpC2 (lthrC::P _{hiper-spank} -Cfp-P3 ern)pD150-CFP/TP → 168 (Erm)DM-055trpC2 (lthrC::P _{hiper-spank} -Cfp-P3 ern)pD150-CFP/TP → 168 (Erm)	168	<i>trpC2</i> , considered <i>wild-type</i> strain	Bacillus Genetic Stock Center		
SWV215 $tpC2$ pheA1 $\Omega(spo0A::kan)$ Xu and Strauch (3)DM-020 $tpC2$ $\Omega(amy:E:P_{xy}/fp:P2 spc)$ pSGDM3 - 168 (Sp)DM-021 $tpC2$ $\Omega(amy:E:P_{xy}/fp:P2 spc)$ pSGDM3 - 168 (Sp)DM-022 $trpC2$ $\Omega(amy:E:P_{xy}/fp:P2 spc)$ $\Omega(thrC:P_{hiper-spank}-cfp-p3 erm)$ pSGDM3, pDP150-CFP - 168 (Srn)DM-023 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP - 168 (Erm)DM-024 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP - 168 (Erm)DM-025 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP + 168 (Erm)DM-026 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 Nt erm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-027 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 Nt erm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-028 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 Nt erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-029 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 Nt erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-029 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 Ct erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-031 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 ct erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-031 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-033 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-034 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-035 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-031 $trpC2$ $\Omega(thrC:P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt - 168 (Erm)DM-033 $trpC2$ $\Omega(th$	MO-101-P	thr ⁻ spo0A ⁻ su ⁺	Mellado et al. (2)		
DM-020 $trpC2 Q(amy E: P_{yr}yfp-p2 spc)$ pSGDM3 - 168 (Sp) DM-021 $trpC2 Q(amy E: P_{yr}yfp-p2 spc)$ pSGDM4 - 168 (Sp) DM-023 $trpC2 Q(amy E: P_{yr}yfp-p2 spc)$ pSGDM3, pDP150-CFP - 168 (Sp) DM-023 $trpC2 Q(atmy E: P_{yr}yfp-p2 spc) Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP - 168 (Erm) DM-024 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP/TP - 168 (Erm) DM-025 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt - 168 (Erm) DM-026 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt - 168 (Erm) DM-027 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP/TP-Nt - 168 (Erm) DM-029 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP/TP-Nt - 168 (Erm) DM-030 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP/TP-L - 168 (Erm) DM-031 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm)$ pDP150-CFP/TP-C+ - 168 (Erm) DM-031 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-p3 erm) Q(tpoC::rpoC-yfp kan)$ pDP150-CFP/TP - 4168 (Erm) DM-033 $trpC2 Q(thr C:: P_{hiper-spank}-cfp-P3 erm) Q(tpoC::rpoC-yfp kan)$ pDP150-CFP/TP - 4168 (Erm) DM-033 $trpC2 Q(thr $	SWV215	trpC2 pheA1 Ω(spo0A::kan)	Xu and Strauch (3)		
DM-021 $trpC2 \Omega(amyE:P_{syr}/fp-p3 spc)$ pSGDM4 168 (Sp)DM-021 $trpC2 \Omega(amyE:P_{syr}/fp-p3 spc)$ pSGDM4 168 (Sp)DM-023 $trpC2 \Omega(amyE:P_{syr}/fp-p3 spc) \Omega(thrC::P_{hiper-spank}-Cfp-p3 erm)$ pDFIS0-CFP 168 (Sp)DM-024 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 erm)$ pDFIS0-CFP 168 (Erm)DM-025 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 erm)$ pDFIS0-CFP/TP 168 (Erm)DM-026 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 Nt erm)$ pDFIS0-CFP/TN-Nt 168 (Erm)DM-027 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 Nt erm)$ pDFIS0-CFP/TP-Nt 168 (Erm)DM-028 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 arm)$ pDFIS0-CFP/TP-Nt 168 (Erm)DM-029 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 arm)$ pDFIS0-CFP/TP-ANt 168 (Erm)DM-028 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)$ pDFIS0-CFP/TP-ANt 168 (Erm)DM-030 $trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cp) \Omega(neo3427)\Delta mreBFormstone et al. (4)DM-031trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)pDFIS0-CFP/TP-ANt 168 (Erm)DM-032trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)pDFIS0-CFP/TP 168 (Erm)DM-033trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)pDFIS0-CFP/TP 168 (Erm)DM-034trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)pDFIS0-CFP/TP 168 (Erm)DM-035trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-p3 cerm)pDFIS0-CFP/TP 168 (Erm)DM-036trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-P3 erm) \Omega(trpC::rpC-yfp kan)pDFIS0-CFP/TP 168 (Erm)DM-037trpC2 \Omega(thrC::P_{hiper-spank}-Cfp-P3 erm) \Omega(trpC::rpC-yfp kan)pDFIS0-$	DM-020	$trpC2 \Omega(amyE::P_{xyr}yfp-p2 spc)$	pSGDM3 \rightarrow 168 (Sp)		
DM-022 $trpC2 \ \Omega(amy \pounds:: P_{xy}: fr p spc)$ $pSG5472 \rightarrow 168 \ (Sp)$ DM-023 $trpC2 \ \Omega(amy \pounds:: P_{xy}: fr p = 2 \ spc) \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pSG5M3, pDP150-CFP \rightarrow 168 \ (Erm)$ DM-024 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP \rightarrow 168 \ (Erm)$ DM-025 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP \rightarrow 168 \ (Erm)$ DM-026 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Nt \rightarrow 168 \ (Erm)$ DM-026 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Nt \rightarrow 168 \ (Erm)$ DM-028 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Nt \rightarrow 168 \ (Erm)$ DM-029 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Nt \rightarrow 168 \ (Erm)$ DM-030 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Att \rightarrow 168 \ (Erm)$ DM-031 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Att \rightarrow 168 \ (Erm)$ DM-032 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP-Att \rightarrow 168 \ (Erm)$ DM-033 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-CFP/TP = MT \rightarrow 168 \ (Erm)$ DM-034 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-TP \rightarrow 168 \ (Erm)$ DM-033 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-TP \rightarrow 168 \ (Erm)$ DM-034 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-TP \rightarrow 168 \ (Erm)$ DM-035 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp = 3 \ erm)$ $pDP150-TP \rightarrow TF \rightarrow 153 \ (Erm)$ DM-036 $trpC2 \ \Omega(thr C:: P_{hiper-spank}-cfp $	DM-021	trpC2 $\Omega(amyE::P_{xyF}yfp-p3 spc)$	pSGDM4 \rightarrow 168 (Sp)		
DM-023 $trpC2 \ \Omega(am/E::P_{xy}') drp-p2 \ spc) \ \Omega(thrC::P_hiper-spank-cfp \ erm)$ pSGDM3, pDP150-CFP - 168 (Sp)DM-024 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ erm)$ pDP150-CFP - 168 (Erm)DM-025 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ erm)$ pDP150-CFP/TP - 168 (Erm)DM-026 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ Herm)$ pDP150-CFP/TP - 168 (Erm)DM-027 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-028 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-029 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-030 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-031 $trpC2 \ \Omega(thrC::P_hiper-spank-cfp \ p3 \ ADC \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-032 $trpC2 \ \Omega(thrC::P_hiper-spank-fp \ p3 \ ADC \ Herm)$ pDP150-CFP/TP-Nt - 168 (Erm)DM-033 $trpC2 \ \Omega(thrC::P_hiper-spank-fp \ p3 \ ADC $	DM-022	trpC2 $\Omega(amyE::P_{xyF}yfp spc)$	pSG5472 → 168 (Sp)		
DM-024trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp erm)$ pDP150-CFP → 168 (Erm)DM-025trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3Nt erm)$ pDP150-CFP/TP- 168 (Erm)DM-026trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt) → 168 (Erm)DM-027trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt) → 168 (Erm)DM-028trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3At erm)$ pDP150-CFP/TP-Nt) → 168 (Erm)DM-029trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3At erm)$ pDP150-CFP/TP-Nt) → 168 (Erm)DM-030trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3Ct erm)$ pDP150-CFP/TP-At) → 168 (Erm)DM-031trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3Ct erm)$ pDP150-CFP/TP-At) → 168 (Erm)DM-032trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3 erm) \Omega(trpoC:rpoC-yfp kan)$ pDP150-CFP/TP-At) → 168 (Erm)DM-031trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-p3 erm) \Omega(trpoC:rpoC-yfp kan)$ pDP150-CFP/TP → 15126 (Erm)DM-033trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-P3 erm) \Omega(trpoC:rpoC-yfp kan)$ pDP150-CFP/TP → B5126 (Erm)DM-033trpC2 $\Omega(thrC:P_h)_{hiper-spank}-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → B5126 (Erm)DM-044MATa trpI-901 leu2-3, 112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HI53James et al. (6)GAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)YNG132P169-4a, pGAD::p3Dervyn et al. (8)YNG64P169-4a, pGAD::p3Dervyn et al. (8)YNG64P169-4a, pGAD::spADervyn et al. (8)YNG64P169-4a, pGBDU::sp2This workYNG129P169-4a, pGBDU::sp2This workYN	DM-023	trpC2 $\Omega(\text{amyE::P}_{xv\Gamma}\text{yfp-p2 spc}) \Omega(\text{thrC::P}_{hiper-spank}\text{-cfp-p3 erm})$	pSGDM3, pDP150-CFP \rightarrow 168 (Sp)		
DM-025trpC2 Ω(thrC::Phiperspank-cfp-p3 erm)pDP150-CFP/TP → 168 (Erm)DM-026trpC2 Ω(thrC::Phiperspank-cfp-p3Nt erm)pDP150-CFP/TP-Nt → 168 (Erm)DM-027trpC2 Ω(thrC::Phiperspank-cfp-p3Nt erm)pDP150-CFP/TP-Nt → 168 (Erm)DM-028trpC2 Ω(thrC::Phiperspank-cfp-p3ANt erm)pDP150-CFP/TP-Nt → 168 (Erm)DM-029trpC2 Ω(thrC::Phiperspank-cfp-p3ANt erm)pDP150-CFP/TP-ANt → 168 (Erm)DM-029trpC2 Ω(thrC::Phiperspank-cfp-p3ANt erm)pDP150-CFP/TP-ANt → 168 (Erm)DM-030trpC2 Ω(thrC::Phiperspank-cfp-p3ANt erm)pDP150-CFP/TP-ANt → 168 (Erm)DM-031trpC2 Ω(amyE::Psyr)tfp-p3 spc) Ω(neo3427)ΔmreB3725 → DM-021 (Erm)DM-032trpC2 Ω(thrC::Phiperspank-gene 3 erm)pDP150-CFP/TP → 168 (Erm)DM-033trpC2 Ω(thrC::Phiperspank-gene 3 erm)pDP150-CFP/TP → 168 (Erm)DM-0400trpC2 Ω(thrC::Phiperspank-cfp-P3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → 15126 (Erm)DM-050trpC2 Ω(thrC::Phiperspank-cfp-P3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → B5126 (Erm)DM-060trpC2 Ω(thrC::Phiperspank-cfp-P3 erm) Ω(rpoC::rpoC-yfp kan)pD150-CFP/TP → B5126 (Erm)DM-051GAL2-ADE2met2::GAL7-lacZsames et al. (6)S. cerevisiaesames et al. (7)P169-4αMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3Noirot-Gros et al. (7)sames et al. (8)YNG3811P169-4α, pGADYNG132P169-4α, pGADYNG4P169-4α, pGAD::scpAYNG5128P169-4α, pGAD::scpAYNG64P169-4α, pGBDUYNG3809 <t< td=""><td>DM-024</td><td>trpC2 Ω(thrC::P_{hiper-spank}-cfp erm)</td><td>pDP150-CFP \rightarrow 168 (Erm)</td></t<>	DM-024	trpC2 Ω(thrC::P _{hiper-spank} -cfp erm)	pDP150-CFP \rightarrow 168 (Erm)		
DM-026 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt → 168 (Erm)DM-027 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3Nt erm)$ pDP150-CFP/TP-Nt) → 168 (Erm)DM-028 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3IMt erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-029 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3ANt erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-030 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3CMt erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-031 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3CMt erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-032 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3 erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-033 $trpC2 \Omega(thrC::P_hiper-spank-gree 3 erm)$ pDP150-CFP/TP-ANt → 168 (Erm)DM-034 $trpC2 \Omega(thrC::P_hiper-spank-gree 3 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-035 $trpC2 \Omega(thrC::P_hiper-spank-gree 3 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-036 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-037 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-040 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-050 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-061 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-062 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-063 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-064 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150	DM-025	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm)	pDP150-CFP/TP \rightarrow 168 (Erm)		
DM-027trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3Ntl erm)pDP150-CFP/TP-Ntl → 168 (Erm)DM-028trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm)pDP150-CFP/TP-I → 168 (Erm)DM-029trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3ANt erm)pDP150-CFP/TP-Ant → 168 (Erm)DM-030trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3Ct erm)pDP150-CFP/TP-Ct → 168 (Erm)3725trpC2 Ω(neo3427)Δmre8Formstone et al. (4)DM-031trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm)pDP150-TP → 168 (Erm)DM-032trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm) Ω(neo3427)Δmre83725 → DM-021 (Erm)DM-033trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-TP → 168 (Erm)DM-033trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → B5126 (Erm)DM-060trpC2 Ω(thrC::P _{hiper-spank} -cfp-P3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → B5126 (Erm)DM-060trpC2 Ω(thrC::P _{hiper-spank} -cfp-VIII PRD1 erm)pDP150-CFP/TP-8-PRD1→ 168 (Sp)S. cerevisiaePI69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3James et al. (6)GAL2-ADE2met2::GAL7-lac2yNG132PI69-4α, pGADNoirot-Gros et al. (7)yNG3811PI69-4α, pGAD::spADervyn et al. (8)yNG64PI69-4α, pGAD::spADervyn et al. (8)yNG12PI69-4α, pGAD::spADervyn et al. (8)yNG12PI69-4a, pGAD::spADervyn et al. (8)yNG12PI69-4a, pGBDU::spADervyn et al. (8)yNG12PI69-4a, pGBDU::spADervyn et al. (8) <td>DM-026</td> <td>trpC2 Ω(thrC::P_{hiper-spank}-cfp-p3Nt erm)</td> <td>pDP150-CFP/TP-Nt \rightarrow 168 (Erm)</td>	DM-026	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3Nt erm)	pDP150-CFP/TP-Nt \rightarrow 168 (Erm)		
DM-028 $trpC2 \Omega(thrC::P_{hiper-spank}-cfp-p3l erm)$ pDP150-CFP/TP-I \rightarrow 168 (Erm)DM-029 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3AN erm)$ pDP150-CFP/TP-ANt \rightarrow 168 (Erm)DM-030 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3Ct erm)$ pDP150-CFP/TP-ANt \rightarrow 168 (Erm)DM-031 $trpC2 \Omega(thrC::P_hiper-spank-cfp-p3Ct erm)$ pDP150-CFP/TP-Ct \rightarrow 168 (Erm)DM-031 $trpC2 \Omega(thrC::P_hiper-spank-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)DM-032 $trpC2 \Omega(thrC::P_hiper-spank-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)DM-033 $trpC2 \Omega(thrC::P_hiper-spank-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)DM-0404 $trpC2 \Omega(thrC::P_hiper-spank-cfp-29 erm) \Omega(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-050 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP \rightarrow DS126 (Erm)DM-060 $trpC2 \Omega(thrC::P_hiper-spank-cfp-VIII PRD1 erm)$ pDP150-CFP/TP \rightarrow BS126 (Erm)S. cerevisiaePI69-4aMATa trpl-901 leu2-3, 112 ura3-52 his3-200 gal4\Delta gal80\Delta LYS2::GAL1-HIS3James et al. (6)S. carevisiaeGAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)YNG132PI69-4a, pGADNoirot-Gros et al. (7)YNG3811PI69-4a, pGADNoirot-Gros et al. (7)YNG3811PI69-4a, pGAD::spaDervyn et al. (8)YNG66PI69-4a, pGAD::spADervyn et al. (8)YNG66PI69-4a, pGBDU::spADervyn et al. (8)YNG66PI69-4a, pGBDU::spADervyn et al. (8)YNG129PI69-4a, pGBDU::spADervyn et al. (8)YNG129PI69-4a, pGBDU::spADervyn et al. (8)YNG74	DM-027	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3Ntl erm)	pDP150-CFP/TP-NtI \rightarrow 168 (Erm)		
DM-029 $trpC2 Ω(thrC::P_hiper-spank-cfp-p3ΔNt erm)$ pDP150-CFP/TP-ΔNt → 168 (Erm)DM-030 $trpC2 Ω(thrC::P_hiper-spank-cfp-p3Ct erm)$ pDP150-CFP/TP-Ct → 168 (Erm)3725 $trpC2 Ω(thrC::P_hiper-spank-cfp-p3 spc) Ω(neo3427)ΔmreB$ Formstone et al. (4)DM-031 $trpC2 Ω(thrC::P_hiper-spank-gene 3 erm)$ pDP150-TP → 168 (Erm)DM-032 $trpC2 Ω(thrC::P_hiper-spank-gene 3 erm)$ pDP150-TP → 168 (Erm)BS126 $trpC2 Ω(thrC::P_hiper-spank-cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP → BS126 (Erm)DM-060 $trpC2 Ω(thrC::P_hiper-spank-cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP → BS126 (Erm)DM-061 $trpC2 Ω(thrC::P_hiper-spank-cfp-P3 erm) Ω(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP → BS126 (Erm)DM-062 $trpC2 Ω(thrC::P_hiper-spank-cfp-Qill PD1 erm)$ pDP150-CFP/TP → BS126 (Erm)DM-064 $trpC2 Ω(thrC::P_hiper-spank-cfp-Qill PD1 erm)$ pD150-CFP/TP3-BPD1→ 168 (Sp)S. cerevisiae	DM-028	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3I erm)	pDP150-CFP/TP-I \rightarrow 168 (Erm)		
DM-030 $trpC2 \ \Omega(thrC::P_{hiper-spank}-cfp-p3Ct erm)$ pDP150-CFP/TP-Ct \rightarrow 168 (Erm)3725 $trpC2 \ \Omega(neo3427) \ ArmeB$ Formstone et al. (4)DM-031 $trpC2 \ \Omega(neo3427) \ ArmeB$ 3725 \rightarrow DM-021 (Erm)DM-032 $trpC2 \ \Omega(thrC::P_{hiper-spank}-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)DM-033 $trpC2 \ \Omega(thrC::P_{hiper-spank}-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)DM-033 $trpC2 \ \Omega(thrC::P_{hiper-spank}-cfp-p3 erm) \ \Omega(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-060 $trpC2 \ \Omega(thrC::P_{hiper-spank}-cfp-p3 erm) \ \Omega(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-060 $trpC2 \ \Omega(thrC::P_{hiper-spank}-cfp-VIII PRD1 erm)$ pDP150-CFP/TP \rightarrow BS126 (Erm)S. cerevisiae	DM-029	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3ΔNt erm)	pDP150-CFP/TP- Δ Nt \rightarrow 168 (Erm)		
3725 $trpC2 \Omega(neo3427)\Delta mreB$ Formstone et al. (4)DM-031 $trpC2 \Omega(amy E: P_{xyF}yfp-p3 spc) \Omega(neo3427)\Delta mreB$ 3725 \rightarrow DM-021 (Erm)DM-032 $trpC2 \Omega(thrC: P_hiper-spank-gene 3 erm)$ pDP150-TP \rightarrow 168 (Erm)BS126 $trpC2 \Omega(trhC::P_niper-spank-gene 3 erm)$ Davies et al. (5)DM-033 $trpC2 \Omega(trhC::P_niper-spank-gene 3 erm)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-043 $trpC2 \Omega(thrC::P_niper-spank-fp-p3 erm) \Omega(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-060 $trpC2 \Omega(thrC::P_niper-spank-fp-VIII PRD1 erm)$ pDP150-CFP/TP \rightarrow BS126 (Erm)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3, 112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3James et al. (6)GAL2-ADE2met2::GAL7-lacZVoirot-Gros et al. (7)GAL2-ADE2met2::GAL7-lacZPJ69-4aMATa trpl-901 leu2-3, 112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3Noirot-Gros et al. (7)yNG132PI69-4a, pGADNoirot-Gros et al. (7)yNG132PI69-4a, pGADDervyn et al. (8)yNG1928PJ69-4a, pGAD::sp3Dervyn et al. (8)yNG64PJ69-4a, pGAD::scpADervyn et al. (8)yNG21PI69-4a, pGBDU::scpADervyn et al. (8)yNG121PJ69-4a, pGBDU::scpADervyn et al. (8)yNG129PJ69-4a, pGBDU::scpADervyn et al. (8)yNG74PJ69-4a, pGBDU::scpADervyn et al. (8)	DM-030	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3Ct erm)	pDP150-CFP/TP-Ct \rightarrow 168 (Erm)		
DM-031trpC2 Ω(amyE::Pxy/ryfp-p3 spc) Ω(neo3427)ΔmreB3725 → DM-021 (Erm)DM-032trpC2 Ω(thrC::Phiperspank-gene 3 erm)pDP150-TP → 168 (Erm)BS126trpC2 Ω(tprC::rpoC-yfp kan)Davies et al. (5)DM-033trpC2 Ω(thrC::Phiperspank-cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → BS126 (Erm)DM-060trpC2 Ω(thrC::Phiperspank-cfp-VIII PRD1 erm)pDP150-CFP/TP → BS126 (Erm)DM-060trpC2 Ω(thrC::Phiperspank-cfp-VIII PRD1 erm)pDP150-CFP/TP8-PRD1→ 168 (Sp)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3 GAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)YNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG124PJ69-4α, pGAD::spADervyn et al. (8)yNG66PJ69-4α, pGAD::spADervyn et al. (8)yNG121PJ69-4α, pGBDUNoirot-Gros et al. (7)yNG3809PJ69-4a, pGBDU::sp2This workyNG129PJ69-4a, pGBDU::sp2This workyNG129PJ69-4a, pGBDU::sp2This workyNG129PJ69-4a, pGBDU::spADervyn et al. (8)yNG74PJ69-4a, pGBDU::spADervyn et al. (8)	3725	trpC2 Ω(neo3427)ΔmreB	Formstone et al. (4)		
DM-032trpC2 Ω(thrC::P _{hiper-spank} -gene 3 erm)pDP150-TP → 168 (Erm)BS126trpC2 Ω(tpoC::rpoC-yfp kan)Davies et al. (5)DM-033trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → BS126 (Erm)DM-060trpC2 Ω(thrC::P _{hiper-spank} -cfp-VIII PRD1 erm)pDP150-CFP/TP → BS126 (Erm)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3James et al. (6)GAL2-ADE2met2::GAL7-lacZPJ69-4αMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3Noirot-Gros et al. (7)GAL2-ADE2met2::GAL7-lacZYNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG3811PJ69-4α, pGADDervyn et al. (8)yNG64PJ69-4α, pGAD::sp3Dervyn et al. (8)yNG66PJ69-4α, pGADDervyn et al. (8)yNG121PJ69-4a, pGBDU::sp2This workyNG129PJ69-4a, pGBDU::sp2This workyNG74PJ69-4a, pGBDU::scpADervyn et al. (8)Dervyn et al. (8)Dervyn et al. (8)	DM-031	trpC2 Ω(amyE::P _{xyr} yfp-p3 spc) Ω(neo3427)ΔmreB	3725 → DM-021 (Erm)		
BS126trpC2 Ω(rpoC::rpoC-yfp kan)Davies et al. (5)DM-033trpC2 Ω(thrC::Phiper-spank-cfp-93 erm) Ω(rpoC::rpoC-yfp kan)pDP150-CFP/TP → BS126 (Erm)DM-060trpC2 Ω(thrC::Phiper-spank-cfp-VIII PRD1 erm)pDP150-CFP/TP-→ BS126 (Erm)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3 GAL2-ADE2met2::GAL7-lacZJames et al. (6)PJ69-4αMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3 GAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)yNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG3811PJ69-4α, pGADDervyn et al. (8)yNG66PJ69-4α, pGAD::spADervyn et al. (8)yNG66PJ69-4α, pGAD::scpADervyn et al. (8)yNG121PJ69-4a, pGBDUNoirot-Gros et al. (7)yNG3809PJ69-4a, pGBDU::p2This workyNG129PJ69-4a, pGBDU::spADervyn et al. (8)yNG74PJ69-4a, pGBDU::scpADervyn et al. (8)	DM-032	trpC2 Ω(thrC::P _{hiper-spank} -gene 3 erm)	pDP150-TP \rightarrow 168 (Erm)		
DM-033 $trpC2 \Omega(thrC::P_{hiper-spank}-cfp-p3 erm) \Omega(rpoC::rpoC-yfp kan)$ pDP150-CFP/TP \rightarrow BS126 (Erm)DM-060 $trpC2 \Omega(thrC::P_{hiper-spank}-cfp-VIII PRD1 erm)$ pDP150-CFP/TP8-PRD1 \rightarrow 168 (Sp)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3James et al. (6)GAL2-ADE2met2::GAL7-lacZJames et al. (7)PJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3Noirot-Gros et al. (7)gAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)Sourt-Gros et al. (7)yNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG3811PJ69-4α, pGADDervyn et al. (8)yNG64PJ69-4α, pGAD::smcDervyn et al. (8)yNG64PJ69-4a, pGAD::scpADervyn et al. (8)yNG121PJ69-4a, pGBDU::p2This workyNG129PJ69-4a, pGBDU::smcDervyn et al. (8)yNG129PJ69-4a, pGBDU::scpADervyn et al. (8)yNG74PJ69-4a, pGBDU::scpADervyn et al. (8)	BS126	trpC2 Ω (rpoC::rpoC-yfp kan)	Davies et al. (5)		
DM-060trpC2 Ω(thrC::P _{hiper-spank} -cfp-VIII PRD1 erm)pDP150-CFP/TP8-PRD1→ 168 (Sp)S. cerevisiaePJ69-4aMATa trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3 GAL2-ADE2met2::GAL7-lacZJames et al. (6)PJ69-4αMATα trpl-901 leu2-3,112 ura3-52 his3-200 gal4∆ gal80∆ LYS2::GAL1-HIS3 GAL2-ADE2met2::GAL7-lacZNoirot-Gros et al. (7)yNG132PJ69-4α, pGADNoirot-Gros et al. (7)yNG3811PJ69-4α, pGAD::p3This workyNG1928PJ69-4α, pGAD::smcDervyn et al. (8)yNG64PJ69-4α, pGAD::scpADervyn et al. (8)yNG65PJ69-4α, pGAD::scpADervyn et al. (8)yNG121PJ69-4a, pGBDU::p2Noirot-Gros et al. (7)yNG129PJ69-4a, pGBDU::p2This workyNG129PJ69-4a, pGBDU::scpADervyn et al. (8)yNG74PJ69-4a, pGBDU::scpADervyn et al. (8)	DM-033	trpC2 Ω(thrC::P _{hiper-spank} -cfp-p3 erm) Ω(rpoC::rpoC-yfp kan)	pDP150-CFP/TP \rightarrow BS126 (Erm)		
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yNG74 PJ69–4a, pGBDU:: <i>scpA</i> Dervyn et al. (8)	vNG129	PJ69–4a, pGBDU::smc	Dervyn et al. (8)		
	yNG74	PJ69–4a, pGBDU::scpA	Dervyn et al. (8)		

Antibiotic resistance gene abbreviations are as follows: *erm*, erythromycin; *kan*, kanamycin; *neo*, neomycin; *spc*, spectinomycin. "X" \rightarrow "Y" indicates that strain Y was transformed with DNA from source X, with selected marker in parentheses. Amp, ampicillin; Em, erythromycin; Sp, spectinomycin.

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Table S2. ϕ 29 phages and plasmids used

TAS PNAS

Phage	Laboratory stock (1) (2) (3)			
φ29 wild-type sus14(1242) sus2(513) sus2(71)				
Plasmid	Relevant features	Reference		
pSG1729	bla amvE3' spc P_{yy} -gfpmut1' amvE5'. N-terminal GFP fusion vector	(3)		
pSG5472	pSG1729 derivative containing <i>vfpmut2</i> gene instead of <i>afpmut1</i>	(4)		
pDP150-CFP/TP8-PRD1	pDP150-CFP-2 containing <i>cfp</i> :: <i>VIII</i> PRD1 fusion	This work		
pSGDM3	pSG5472 containing vfp::p2 fusion	(4)		
pSGDM5	pSG5472 containing vfp::p3 fusion	This work		
pDR200	Vector containing cfp (Bs) optimized for B. subtilis expression	(5)		
pDP150	pDR111 derivative thrC integrating vector containing Phyper-spank	(6)		
pDP150-CFP	pDP150 containing cfp (Bs)	This work		
pDP150-CFP-2	pDP150-CFP containing a Spel site at the MCS	This work		
pDP150-CFP/TP	pDP150-CFP-2 containing cfp::p3 fusion	This work		
pDP150-CFP/TP-Nt	pDP150-CFP-2 containing cfp::p3-Nt fusion	This work		
pDP150-CFP/TP-Ntl	pDP150-CFP-2 containing cfp::p3-Ntl fusion	This work		
pDP150-CFP/TP-I	pDP150-CFP-2 containing <i>cfp::p3-I</i> fusion	This work		
pDP150-CFP/TP-∆Nt	pDP150-CFP-2 containing <i>cfp::p3-bNt</i> fusion	This work		
pDP150-CFP/TP-Ct	pDP150-CFP-2 containing cfp::p3-Ct fusion	This work		
pDP150-TP	pDP150 containing ϕ 29 <i>gene 3</i>	This work		
pET-28b(+)	E. coli expression vector for protein purification	Novagen		
рТ7-3	E. coli expression vector for protein purification	(7)		
рТ7-3-ТР	pT7-3 vector containing gene 3	8)		
pET-TP/Nt	pET-28b(+) containing gene 3-Nt region	This work		
pET-TP/Ntl	pET-28b(+) containing gene 3-Ntl region	(8)		
pET-TP/ΔNt	pET-28b(+) containing gene 3-ICt region	(8)		
pET-TP/Ct	pET-28b(+) containing gene 3-Ct region	(8)		

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Table S3. Oligonucleotides used

PNAS PNAS

Oligonucleotide	Sequence
YFP-TP_U	5'-gat aac tcg aga tgg cga gaa gtc cac gta tac gc-3'
YFP-TP_L	5'-ttt aag aat tcc tag aac ccc ttt aag ctt aga tca aag tc-3'
CFP(BS)_U	5'-cgt gat taa aag ctt aca taa gga gga ac-3'
CFP(BS)_L	5'-tta agg agc tag cct tat aaa gtt cgt cca tgc caa gtg taa tgc-3'
150-Spel-1	5'-gca agc taa ttc ggt gga aac tag ttc atc att tcc ttc cga aaa aac gg-3'
150-Spel-2	5'-ccg ttt ttt cgg aag gaa atg atg aac tag ttt cca ccg aat tag ctt gc-3'
TP_1	5'-cat gcg cta gcg aaa gga gat aa cgc aac atg gcg aga ag-3'
TP_2	5'-ttt aag cat gcc tag aac ccc ttt aag ctt aga tc-3'
TP_U	5'-gag atg cat gca cat ggc gag aag tcc acg tat-3'
TP_L	5'-ttt aaa cta gtc tag aac ccc ttt aag ctt a-3'
TP-Nt_L	5'-ttt tca cta gtt tat cac ata tta gca cgg tta gtg aaa gag g-3'
TP-NtI_L	5'-ttc tta cta gtt tac taa ggg tct gtt ctc atc tcc atg c-3'
TP-I_U	5'-taa ccg cat gca tat gcg tta tca gtt cga aaa g-3'
TP-Ct_U	5'-gag aag cat gcc tca gta tta tga aaa gaa aat gat aca g-3'
TP-Ncol_U	5'-taa cgc acc atg gcg aga agt cca cgt ata cgc att aag g-3'
TP-NtI-NotI_L	5'-ctt ttc agc ggc cgc agg gtc tgt tct cat ctc cat gct t-3'
TP-Ndel_U	5'-aac gcc ata tgg cga gaa gtc cac gta tac-3'
TP-Nt-NotI_L	5'-tct ttg cgg ccg ctt atc aca tat tag cac ggt tag tga aaa gag g-3'
TP-I-Ndel_U	5'-cgg cag cca tat gcg tta tca gtt cg-3'
TP-BamHI_L	5'-cgc ggg atc cgg agc cta gaa cc-3'
TP-Ct-Ndel_U	5'-gcg cgc ata tgt att atg aaa aga aaa tg-3'
yshC_U	5'-ccg cgg atc cca tca ttt tac ggg-3'
yshC_L	5'-ggg tcg aca ctt cct gtc cgc ttt cac g-3'
R-OUT-SUPER	5'-aaa tag att ttc ttt ctt ggc tac-3'
R-25	5'-aaa gta ggg tac agc gac aac ata c-3'
TP8-R	5'-cgg aag gca tgc tca tgg cga aga aaa aac cag tag aa-3'
TP8-L	5'-acg gcg act agt tca tta aac ccc ctt gct gcc ata gcc gcg-3'