#### **Supporting information**

**Fig. S1.** RNA-Seq analyses of *spoIID*, *spoIIP*, and *spoIIM* transcription in sporulating JIR8094 strains visualized using the Integrated Genome Viewer software

Fig. S2. Construction of mutants lacking engulfment-related genes.

Fig. S3. Comparison of heat-resistant spore formation by  $\Delta IIQ$  grown in broth vs. on plates.

Fig. S4. Read-through transcription of *spoIIP* increases IIP levels in *C. difficile*.

Fig. S5. Fluorescence microscopy analyses of engulfment progression.

**Fig. S6.** Severe engulfment and coat localization defects in  $\Delta IID$ ,  $\Delta IIP$ ,  $\Delta IIM$  and  $\Delta IIQ$  mutant derivatives.

**Fig. S7.** Complementation of  $\Delta sipL$  by a construct encoding SipL-mCherry.

Fig. S8. Western blot analyses of mCherry fusion proteins used in localization studies.

Fig. S9. Localization of the outer basement layer protein, SipL, in engulfment mutants.

Supplementary Text S1 – Description of cloning of plasmid constructs.

**Supplementary Table S1** – *C. difficile* and *E. coli* strains used in this study.

Supplementary Table S2 – Primers used in this study.

**Supplementary Table S3 -** Sporulation heat resistance ratios determined for all strains across all replicates.

Α.





С.

250 bp



Fig. S1. RNA-Seq analyses of *spoIID*, *spoIIP*, and *spoIIM* transcription in sporulating JIR8094 strains visualized using the Integrated Genome Viewer software (Robinson *et al.*, 2011). Histograms of RNA sequence reads (grey) in wildtype JIR8094 and sporulation mutants created using Targetron-based gene disruption (Heap *et al.*, 2007). The reads mapped to these loci are shown below the histograms. *spo0A* encodes a master transcriptional activator required for inducing sporulation; *sigF* encodes  $\sigma^F$ , a forespore-specific sigma factor that is necessary for

activating the forespore-specific sigma factor,  $\sigma^{G}$ , and contributes to the activation of  $\sigma^{E}$ , a mother cell-specific sigma factor encoded by *sigE* (Fimlaid & Shen, 2015).  $\sigma^{E}$  is necessary for transcriptional activation of *sigK*, which encodes the mother cell-specific sigma factor,  $\sigma^{K}$ (Pishdadian *et al.*, 2015, Saujet *et al.*, 2013). Genes are shown as colored rectangles, with the chevron indicating the direction of transcription. Bent arrows represent transcriptional start sites. Promoters whose sporulation sigma factor regulation had been previously established are shown (Saujet *et al.*, 2013). (A) *spoIIP* is encoded downstream of *gpr*; (B) *spoIID* is encoded upstream of *spoIIQ*; and (C) *spoIIM* (*CD1221*) is encoded upstream of a XerC recombinase gene. The histograms shown are representative of the results obtained from three independent biological replicates.







Fig. S2. Construction of mutants lacking engulfment-related genes. (A) Schematic of gene

deletions. Flanking primers bind outside the region of homology cloned into pMTL-YN3 and were used, along with an internal red primer, to confirm the deletion of *IIP* and *IIM* in multigene deletion strains (B). (C) Colony PCR of first generation single mutants,  $\Delta IID$ ,  $\Delta IIP$ ,  $\Delta IIM$ , and  $\Delta IIQ$ , and the  $\Delta IIDQ$  double mutant using either the flanking primer pair or the 5' flanking primer with the 3' internal primer to assess strain purity.



Fig. S3. Comparison of heat-resistant spore formation by  $\Delta HQ$  grown in broth vs. on

**plates.** Phase-contrast microscopy analyses of the indicated strains after 24 hrs of sporulation. The ratio of heat-resistant spores measured for each strain from 23 hr sporulating cultures was determined based on three independent biological replicates. Statistical significance relative to wild type was determined using a one-way ANOVA and Tukey's test. Scale bars represent 5  $\mu$ m. The limit of detection of the assay is 10<sup>-6</sup>.



Fig. S4. Read-through transcription of *spoIIP* increases IIP levels in *C. difficile*. (A) Schematic of *spoIIP* complementation constructs encoding Glu309A mutations in IIP. Bent arrows represent transcriptional start sites, although only the *gpr* transcriptional start site has been mapped; dashed lines indicate likely position of the proximal promoter based on results of this figure. (B) Western blot analyses of IIP in  $\Delta spoIIP$  complementation constructs with *spoIIP* deletion and targetron (*IIP*<sup>-</sup>) mutant controls. Three separate isoforms were detected for IIP in wild type: full-length (IIP), truncated IIP (t-IIP), and cleaved IIP (c-IIP). The predicted MW of IIP is 38 kDa, and the MW of IIP lacking its signal peptide is 35 kDa. The asterisks denote nonspecific proteins recognized by the polyclonal anti-IIP antibodies. Spo0A was used as a proxy for measuring sporulation induction (Dembek *et al.*, 2017, Putnam *et al.*, 2013). The western blots shown are representative of the results from three independent biological replicates. Heat resistance (H.R.) efficiencies were determined from 23 hr cultures and represent the mean and

standard deviation for a given strain relative to wild type based on a minimum of three independent biological replicates. The limit of detection of the assay is  $10^{-6}$ .



→ Incomplete engulfment (Hoechst-positive forespore, polar septum)

- → Asymmteric engulfment
- → Engulfment complete
- Detached forespores

Fig. S5. Fluorescence microscopy analyses of engulfment. Wild type and single gene engulfment mutants were grown on sporulation media for 24 hrs and evaluated by live differential interference contrast (DIC) and fluorescence microscopy. Hoechst was used to stain the nucleoid (blue), and FM4-64 was used to stain membranes (red). Fully engulfed cells exclude the Hoechst stain from the forespore (Fimlaid *et al.*, 2015, Sharp & Pogliano, 1999), while engulfed cells that complete membrane fission exclude FM4-64 (Doan *et al.*, 2013). Yellow arrows designate cells that have completed engulfment, green arrows highlight forepores undergoing asymmetric engulfment, and pink arrows indicate detached forespores observed in  $\Delta IIP$ . Sporulation was arrested at asymmetric division in  $\Delta IIP$ , while  $\Delta IIQ$  and  $\Delta IID$  forespores exhibited greater curvature of the forespore membrane;  $\Delta IID$  forespores exhibited asymmetric engulfment at higher frequencies than wild type. The percentage of cells that completed engulfment or exhibited asymmetric engulfment is shown based on analyses of >200 cells.

		Engulfed	Coat Bearding	Double Beard	Cytosolic
WT	( so )	100%	0%	0%	0%
ΔIID	0.20	0%	86%	0%	4%
∆IIP		0%	82%	32%	12%
ΔIIM		86%	16%	2%	4%
ΔIIQ		2%	70%	12%	22%
ΔIIDQ	6	0%	70%	18%	30%
ΔIIDP		0%	82%	16%	14%
ΔIIPQ		0%	73%	29%	40%
∆IIDPQ		0%	80%	34%	20%
∆IIDPM		0%	72%	24%	22%
ΔIIDPMQ		0%	72%	34%	26%
	Properly localized coat				
	<ul> <li>Mislocalized coat (bearding)</li> </ul>				

Double beard

Leading edge of engulfment

Fig. S6. Severe engulfment and coat localization defects in  $\Delta IID$ ,  $\Delta IIP$ ,  $\Delta IIM$  and  $\Delta IIQ$  mutant derivatives. Transmission electron microscopy (TEM) analyses of wildtype  $630\Delta erm$  and engulfment mutants after 23 hrs of sporulation induction. Scale bars represent 500 nm. Yellow arrows mark properly localized coat, and pink arrows mark mislocalized coat. Blue arrows mark the leading edge of the engulfing membranes in cells that fail to complete engulfment. The percentages shown are based on analyses of 50 cells for each strain with visible signs of sporulation from the same single biological replicate shown in Fig. 4 with the exception of  $\Delta IIPQ$ . Coat bearding refers to polymerized coat that sloughs off from the forespore. "Double Beard" refers to when two layers of polymerized coat were observed such as in the  $\Delta IIDPM$  and  $\Delta IIDPMQ$  mutants (green arrows). Cytosolic refers to polymerized coat visible in the mother cell cytosol, i.e. detached from the forespore.



→ Phase Bright Spore

Fig. S7. Complementation of  $\Delta sipL$  with a *sipL-mCherry* construct. Phase-contrast microscopy analyses of wild type  $630\Delta erm$ ,  $\Delta spo0A$  ( $\Delta 0A$ ), and  $\Delta sipL$  strains 21 hrs after sporulation induction. Yellow arrows mark example phase-bright forespores, while pink arrows demarcate regions suspected to be mislocalized coat based on previous studies (Fimlaid *et al.*, 2015, Ribis *et al.*, 2017). Heat resistance (H.R.) efficiencies were determined from 23 hr cultures and represent the mean and standard deviation for a given strain relative to wild type based on a minimum of three independent biological replicates. The limit of detection of the assay is  $10^{-6}$ .



Fig. S8. Western blot analyses of mCherry fusion proteins used in localization studies.

Antibodies to mCherry (mC), IVA, SipL and CotE were used as indicated. Asterisks indicate non-specific bands. Partial processing of mCherry-IVA releases free mCherry as previously reported (Ribis *et al.*, 2017). Several isoforms of SipL and SipL-mCherry are detected, most that run higher than expected. The western blots shown are representative of the results from three independent biological replicates.



Fig. S9. Localization of the outer basement layer protein, SipL, in engulfment mutants. Fluorescence microscopy analyses of  $\Delta sipL$ ,  $\Delta sipL\Delta IIM$ , and  $\Delta sipL\Delta IIP$  cells producing SipLmCherry fusions at 23 hrs post sporulation induction. Phase-contrast (phase) microscopy was used to visualize sporulating cells. Hoechst staining of the nucleoid is shown in blue, and

mCherry fluorescence is shown in red. Engulfment completion excludes Hoechst from the forespore (Pogliano *et al.*, 1999). The merge of Hoechst and mCherry (top) and phase-contrast and mCherry (bottom) is shown. Yellow arrows denote encasement of the forespore; green arrows highlight staining along the presumed polar septum based on Hoechst labeling; and purple arrows highlight punctate foci. Schematics depicting the primary localization pattern of SipL-mCherry, and the percentage of a given phenotype is shown as is the total number of cells analyzed.

## Supplementary Text S1: Cloning of plasmid constructs

#### **1. Deletion constructs**

**pMTL-YN3**  $\Delta IID$ . Primer pair #1959 and 1961 was used to amplify the region 940 bp upstream of *spoIID* primer pair #1960 and 1962 to amplify the region 921 bp down stream of *spoIID*. The resulting PCR products were used in a PCR SOE (Horton *et al.*, 1989) with the flanking primers #1959 and 1962 to generate a fragment than encodes an in-frame deletion of *spoIID* where the first 12 codons are linked to the last 22 codons. The PCR SOE fragment was digested with AscI and SbfI and ligated to pMTL-YN3 digested with the same enzymes.

**pMTL-YN3-** $\Delta$ *IIP*. Primer pair #1941 and 1943 was used to amplify the region 1000 bp upstream of *spoIIP*; primer pair #1942 and 1944 was used to amplify the region 1000 bp downstream of *spoIIP*. The resulting PCR products were used in a PCR SOE with the flanking primers #1941 and 1944 to generate a fragment than encodes an in-frame deletion of *spoIIP* where the first 21 codons are linked to the last 12 codons. The PCR SOE fragment was digested with AscI and SbfI and ligated to pMTL-YN3 digested with the same enzymes.

**pMTL-YN3-**Δ*IIM*. Primer pair #1999 and 2002 was used to amplify the region 1001 bp upstream of *spoIIM* primer pair #2001 and 2000 to amplify the region 1059 bp downstream of *spoIIM*. The resulting PCR products were used in a PCR SOE with the flanking primers #1999 and 2000 to generate a fragment than encodes an in-frame deletion of *spoIIM* where the first 22 codons are linked to the last 11 codons. The PCR SOE fragment was digested with AscI and SbfI and ligated to pMTL-YN3 digested with the same enzymes.

**pMTL-YN3-**Δ*IIQ*. Primer pair #1965 and 1967 was used to amplify the region 1025 bp upstream of *spoIIQ* primer pair #1966 and 1968 to amplify the region 975 bp downstream of *spoIIQ*. The resulting PCR products were used in a PCR SOE with the flanking primers #1965 and 1968 to generate a fragment than encodes an in-frame deletion of *spoIIQ* where the first 28 codons are linked to the last 22 codons. The PCR SOE fragment was digested with AscI and SbfI and ligated to pMTL-YN3 digested with the same enzymes.

**pMTL-YN3-**Δ*IIDQ*. Primer pair #1959 and 1974 was used to amplify the region 1025 bp upstream of *spoIIDQ* primer pair #1973 and 1968 to amplify the region 975 bp downstream of *spoIIDQ*. The resulting PCR products were used in a PCR SOE with the flanking primers #1959 and 1968 to generate a fragment than encodes an in-frame deletion of *spoIIDQ* where the first 28 codons of *spoIID* are linked to the last 22 codons of *spoIIQ*. The PCR SOE fragment was digested with AscI and SbfI and ligated to pMTL-YN3 digested with the same enzymes.

**pMTL-YN3-** $\Delta$ *sipL*. Primer pair #1995 and 1760 was used to amplify the region 999 bp upstream of *sipL*. primer pair #1759 and 2088 to amplify the region 591 bp down stream of *sipL*. The resulting PCR products were cloned into pMTL-YN3 digested with AscI and SbfI using Gibson assembly (Gibson *et al.*, 2009). The construct encodes an in-frame deletion of *sipL* where the first 15 codons of *sipL* are linked to the last 15 codons.

#### 2. Targetron construct

To create the *spoIIP* targetron construct, a modified plasmid containing the retargeting group II intron, pCE245 (a gift from C. Ellermeier, University of Iowa) was used as a template. Primers #1487, 1488, 1489, and 532 (EBS universal primer, Sigma Aldrich) were used.

#### 3. Complementation constructs

**pMTL-YN1C**-*spoIID*. Primer pair #1572 and 1573 were used to amplify the region spanning 246 bp upstream of *spoIID* and 35 bp downstream of *spoIID*. The resulting product was digested with NotI and XhoI then ligated into pMTL-YN1C digested with the same enzymes.

**pMTL-YN1C**-*spoIID*<sub>E101A</sub>. Primer pair #2231 and 2380 and primer pair #2379 and 2378 were used to amplify regions spanning 246 bp upstream of *spoIID* and 35 bp downstream of *spoIID*. Primers 2379 and 2380 encode a Glu309Ala mutation in *spoIIP* that inactivates its catalytic function. The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*spoIIP*<sub>E309A</sub>. Primer pair #2481 and 2382 and primer pair #2381 and 2383 were used to amplify regions spanning 269 bp upstream of *spoIIP* and 282 bp downstream of *spoIIP* (includes  $CD630_{24860}$ ). Primers 2382 and 2383 encode a Glu309Ala mutation in *spoIIP* that inactivates its catalytic function. The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*gpr-spoIIP*<sub>E309A</sub>. Primer pair #2352 and 2382 and primer pair #2381 and 2383 were used to amplify regions spanning 204 bp upstream of *gpr* and 282 bp downstream of *spoIIP* (includes  $CD630_24860$ ). Primers 2382 and 2383 encode a Glu309Ala mutation in *spoIIP* that inactivates its catalytic function. The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*spoIIQ*. Primer pair #1177 and 1178 were used to amplify the region spanning 106 bp upstream of *spoIIQ* and 9 bp downstream of *spoIIQ*. The resulting product was digested with NotI and XhoI then ligated into pMTL-YN1C digested with the same enzymes.

**pMTL-YN1C**-*spoIIQ*<sub>H120A</sub>. Primer pair #2449 and 1851 and primer pair #1850 and 2232 were used to amplify regions spanning 106 bp upstream of *spoIIQ* and 9 bp downstream of *spoIIQ* (includes  $CD630_24860$ ). Primers 2382 and 2383 encode a His120Ala mutation in *spoIIQ* that disrupts binding to Zn<sup>2+</sup> and would inactivate IIQ's putative endopeptidase activity. The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*spoIID*. Primer pair #2231 and 2232 were used to amplify the region spanning 246 bp upstream of *spoIID* and 9 bp downstream of *spoIIQ*. The resulting product was cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*spoIIM*. Primer pair #1576 and 1577 were used to amplify the region spanning 231 bp upstream of *spoIIM* and 82 bp downstream of *spoIIM*. The resulting product was digested with NotI and XhoI then ligated into pMTL-YN1C digested with the same enzymes.

**pMTL-YN1C**-*sipL*. Plasmid pMTL83151-*sipL* (Putnam *et al.*, 2013) was digested with NotI/XhoI, and the resulting *sipL* fragment (include 318 bp upstream and 102 bp downstream of *sipL* was gel-purified and ligated into pMTL-YN1C digested with the same enzymes.

### 4. mCherry constructs

**pMTL-YN1C**-*sipL-mCherry*. Primer pair #2165 and 2212 was used to amplify the *sipL* gene including 318 bp of its upstream region. Primer pair #2211 and 2133 was used to amplify the *mCherry* gene with an alanine codon linker using the codon-optimized *mCherry* construct from Ransom *et al.* as the template (Ransom *et al.*, 2015). The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

**pMTL-YN1C**-*cotE-mCherry*. Primer pair #2268 and 2264 was used to amplify the *cotE* gene including 228 bp of its upstream region. Primer pair #2263 and 2133 was used to amplify the *mCherry* gene with an alanine codon linker using the codon-optimized *mCherry* construct from **Ransom** *et al.* as the template. The PCR products were cloned into pMTL-YN1C digested with NotI and XhoI using Gibson assembly.

### 5. pET expression constructs

**pET21a**-*spoIID*<sub> $\Delta 27aa$ </sub>. Primer pair #1631 and 1632 were used to amplify *spoIID* lacking its first 27 codons and its stop codon. The resulting product was digested with NheI and XhoI then ligated into pET21a digested with the same enzymes.

**pET28a**-*spoIIP*<sub> $\Delta 27aa$ </sub>. Primer pair #2297 and 2316 were used to amplify *spoIIP* lacking its first 27 codons and its stop codon. The resulting product was cloned into pET28a digested with NcoI and XhoI using Gibson assembly.

# Supplementary Table S1. Strains used in this study

C. difficite strains - 630 $\Delta erm$ (Ng et al.,756630 $\Delta erm Appr E$ erm-sensitive derivate of 630 with a deletion in $pyrE$ 2013)807630 $\Delta erm Appr E$ MID630 $\Delta erm Appr E$ with spoID (CD024) deletedThis study817630 $\Delta erm Appr E$ MID630 $\Delta erm Appr E$ with spoID (CD012) deletedThis study818630 $\Delta erm AlpyrE$ MID630 $\Delta erm Appr E$ with spoID (CD012) deletedThis study818630 $\Delta erm Alport E$ MIDP630 $\Delta erm Alpy E$ with spoID in the pyrE locusThis study818630 $\Delta erm Alport MID$ 630 $\Delta erm Alport MID$ gold $\Delta erm Alport MID$ (Donnelly et al., 2017)846630 $\Delta erm Appr E$ MIDP630 $\Delta erm Appr E$ with spoID in the pyrE nestored(Donnelly et al., 2017)854630 $\Delta erm Appr E$ MIDP630 $\Delta erm Appr E$ with spoID poID, and spoIDQ deletedThis study907630 $\Delta erm Alpyr E$ MIDP630 $\Delta erm Alpyr E$ with spoID in the pyrE restoredThis study913630 $\Delta erm Alpr P$ 630 $\Delta erm AlDr M$ This study914630 $\Delta erm AlDP P$ 630 $\Delta erm AlDr M The pyrE restoredThis study915630 \Delta erm AlDP P630 \Delta erm AlDR M The pyrE restoredThis study916630 \Delta erm Alpr P630 \Delta erm AlDR M The pyrE restoredThis study917630 \Delta erm Alpr P =630 \Delta erm Alpr P E restoredThis study918630 \Delta erm Alpr P =630 \Delta erm Alpr P =Fits study919630 \Delta erm Alpr P =630 \Delta erm Alpr P =Fits study9116630 \Delta erm Alpr P =630$	Strain #	Strain name	Relevant genotype or features	Source/reference
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826       630Aerm AJQ/UIQ       630AermAJQ/With spolID-spolIQ deleted       This study         838       630Aerm AII/DIIIQ       630AermAJIQ with spolID in the pyrE locus       This study         846       630Aerm AIID       630AermAIID with spolID in the pyrE locus       This study         847       630AermAppre AIIDPQ       630AermAIIDP       630AermAIIDAIIQ with pyrE restored       This study         919       630AermAIIDPQ-P       630AermAIIDAIIQ with pyrE restored       This study       103       630AermAppre Aippre AipII ABIAPAIQ with pyrE restored       This study         926       630AermAppre AipP       630AermAppre AipII ABIAPAIQ with pyrE restored       This study         936       630AermAppre AipP       630AermAppre AipPP with pyr	819	$630\Delta erm\Delta pyrE \Delta IIQ$	$630\Delta erm\Delta pyrE$ with spoIIQ (CD0125) deleted	This study
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846 $630 Aerm.p$ $erm.sensitive derivate of 630 with pyrE restored(Donnelly etal., 2017)849630 Aerm Aspo0A-p630 AermAspo0A with pyrE restored(Donnelly etal., 2017)854630 AermAspyrE AIIM630 AermAsprE with spoIID, spoIP, and spoIIQ deletedThis study907630 Aerm AID-p630 AermAID with pyrE restoredThis study918630 Aerm AID-p630 AermAID with pyrE restoredThis study919630 Aerm AID_P_p630 AermAID W with pyrE restoredThis study916630 Aerm AID_P_p630 AermAID W with pyrE restoredThis study922630 Aerm AID_P_p630 AermAID W with pyrE restoredThis study924630 Aerm AID_P_p630 AermAID W with pyrE restoredThis study925630 AermAIDMIW630 AermAID W with pyrE restoredThis study926630 AermApyrE AsipL630 AermApyrE with sipL deletedThis study936630 AermApyrE AIIDP630 AermApyrE with sipL deletedThis study937630 AermApyrE AIIDP630 AermApyrE with spOID, spoIP, spoIIQ, and spoIIM deletedThis study938630 AermApyrE AIIDP A630 AermApyrE with spOID, spoIP, spoIIQ, and spOIIM deletedThis study937630 AermApyrE AIIDPA630 AermApyrE with spOID, ad spoIIM deletedThis study938630 AermAIDPAP_P630 AermApyrE with spOID, ad spOIIM deletedThis study939630 AermAIDPAP_P630 AermApyrE with spOID and$	838	$630\Delta erm \Delta IID/IID$	$630\Delta erm\Delta IID$ with <i>spoIID</i> in the <i>pyrE</i> locus	This study
849 $630 \Delta erm \Delta spo0A \cdot p$ $630 \Delta erm \Delta spo1A$ with $pyrE$ restored(Donnelly et al., 2017)854 $630 \Delta erm \Delta pyrE$ $\Lambda IIDPQ$ $630 \Delta erm \Delta pyrE$ with $spoIID$ , $spoIIP$ , and $spoIIQ$ deletedThis study907 $630 \Delta erm \Delta IID-p$ $630 \Delta erm \Delta IID-p$ $630 \Delta erm \Delta IID vith pyrE restoredThis study918630 \Delta erm \Delta IID-p630 \Delta erm \Delta IID p630 \Delta erm \Delta IID p630 \Delta erm \Delta IID p919630 \Delta erm \Delta IID p630 \Delta erm \Delta IID p630 \Delta erm \Delta IID p630 \Delta erm \Delta IID p916630 \Delta erm \Delta IID p630 \Delta erm \Delta IID IP p630 \Delta erm \Delta IID IP p630 \Delta erm \Delta IID Vith pyrE restoredThis study922630 \Delta erm \Delta IID P_p630 \Delta erm \Delta IID AIP AIP Q with pyrE restoredThis study946630 \Delta erm \Delta AipI P_p630 \Delta erm Ain Norkip V with sipL deletedThis study910630 \Delta erm \Delta xipL p630 \Delta erm AipyrE with sipL deletedThis study9110630 \Delta erm \Delta xipL p630 \Delta erm AipyrE with sipL deletedThis study913630 \Delta erm AipyrE AIID PMQ630 \Delta erm AipyrE with spoIID, spoIIP, spoIIQ, and spoIIM deletedThis study914630 \Delta erm AiDP-p630 \Delta erm AiDPA-p630 \Delta erm AiDA erm AiID AIP AID $	846	630∆ <i>erm-</i> p	erm-sensitive derivate of 630 with pyrE restored	(Donnelly <i>et al.</i> , 2017)
854630AermApyrE AIIDPQ630AermApyrE with spoIID, spoIIP, and spoIIQ deletedThis study907630Aerm AIID-p630AermAID with spoIID (CD1221) deletedThis study918630Aerm AIID-p630AermAID with pyrE restoredThis study918630Aerm AIID-p630AermAID with pyrE restoredThis study919630Aerm AIID-p630AermAID with pyrE restoredThis study919630Aerm AIIDQ-p630AermAIDW with pyrE restoredThis study922630Aerm AIIDIM630AermAIIDAIIQ with pyrE restoredThis study923630Aerm AIIDIM630AermAIIDAIM with IM in the pyrE locusThis study910630AermAiIDAIM with IM in the pyrE locusThis study9101630AermAipyrE AsipL630AermApyrE with spiL deletedThis study9103630AermApyrE AIIDPAQ630AermApyrE with spoIID, spoIIP, spoIIQ, and spoIIM deletedThis study9104630AermApyrE AIIDPAQ630AermApyrE with spoIID, spoIID, spoIIP, and spoIIM deletedThis study9105630AermApyrE AIIDPA630AermApyrE with spoIID, spoIIP, spoIIQ, and spoIIM deletedThis study9105630AermAIDAIPATH630AermAIDAIIPAIM with pyrE restoredThis study9105630AermAIDPH-p630AermAIDAIIPAIM with pyrE restoredThis study9116630AermAIDPHQ-p630AermAIDAIDAIMAIM with pyrE restoredThis study91105630AermAIDPHQ-p630AermAIDAIDAIMAIQ with pyrE restoredThis study91116630AermAIDPHQ-p630AermAIDAIDAIMAIM with pyrE restoredThis study <tr< td=""><td>849</td><td>630∆<i>erm</i> ∆<i>spo0A</i>-p</td><td><math>630\Delta erm\Delta spo0A</math> with <i>pyrE</i> restored</td><td>(Donnelly <i>et al.</i>, 2017)</td></tr<>	849	630∆ <i>erm</i> ∆ <i>spo0A</i> -p	$630\Delta erm\Delta spo0A$ with <i>pyrE</i> restored	(Donnelly <i>et al.</i> , 2017)
907 $630 \Delta erm \Delta prE \Delta IIM$ $630 \Delta erm \Delta prE$ with $spoIIM$ ( $CD1221$ ) deletedThis study910 $630 \Delta erm \Delta IID$ -p $630 \Delta erm \Delta IID$ with $prE$ restoredThis study913 $630 \Delta erm \Delta IID_P$ $630 \Delta erm \Delta IID$ with $prE$ restoredThis study914 $630 \Delta erm \Delta IID_P$ $630 \Delta erm \Delta IID P_P$ $630 \Delta erm \Delta IID P_P$ 915 $630 \Delta erm \Delta IIDP_P$ $630 \Delta erm \Delta IID IP_P AID$ $930 \Delta erm \Delta IID P_P$ 926 $630 \Delta erm \Delta IIDP_P$ $630 \Delta erm \Delta IID AIP AID$ $930 \Delta erm \Delta IID P_P$ 937 $630 \Delta erm \Delta IIDP_P$ $630 \Delta erm \Delta IID AIP AID$ $930 \Delta erm \Delta IID AIP AID$ 946 $630 \Delta erm \Delta IIDP_P$ $630 \Delta erm \Delta IID AIP AID$ $930 \Delta erm \Delta IID AIP AID$ 947 $630 \Delta erm \Delta IIDP_P$ $630 \Delta erm \Delta IID AIP AID$ $1111 M III III IIII IIII IIII IIII IIII$	854	$630\Delta erm\Delta pyrE \Delta IIDPQ$	$630\Delta erm\Delta pyrE$ with spoIID, spoIIP, and spoIIQ deleted	This study
910 $630 \Delta erm \Delta IID$ -p $630 \Delta erm \Delta IID$ with $pyrE$ restoredThis study913 $630 \Delta erm \Delta IID$ -p $630 \Delta erm \Delta IID$ with $pyrE$ restoredThis study914 $630 \Delta erm \Delta IID Q$ -p $630 \Delta erm \Delta IID Q$ -p $630 \Delta erm \Delta IID Q$ -p915 $630 \Delta erm \Delta IID Q$ -p $630 \Delta erm \Delta IID AID Q$ -Mit pyrE restoredThis study916 $630 \Delta erm \Delta IID Q$ -p $630 \Delta erm \Delta IID AID Q$ -Mit pyrE restoredThis study917 $630 \Delta erm \Delta IID Q$ -p $630 \Delta erm \Delta IID AID Q$ -Mit pyrE restoredThis study918 $630 \Delta erm \Delta Aip Q$ -p $630 \Delta erm \Delta pyrE$ with $sipL$ deletedThis study1003 $630 \Delta erm \Delta sipL-p$ $630 \Delta erm \Delta pyrE$ with $sipL$ ideletedThis study1016 $630 \Delta erm \Delta pyrE \Delta SipL$ $630 \Delta erm \Delta pyrE$ with $sipL$ ideletedThis study1017 $630 \Delta erm \Delta pyrE \Delta SipL$ $630 \Delta erm \Delta pyrE$ with $spolID$ , $spolIP$ , $spolIQ$ , and $spoIIM$ deletedThis study1018 $630 \Delta erm \Delta pyrE$ AIID PH $630 \Delta erm \Delta pyrE$ with $spoID$ , $spoIIP$ , $spoIIQ$ and $spoIIM$ deletedThis study1102 $630 \Delta erm \Delta IID PhO_p$ $630 \Delta erm \Delta IID AIIP AIM DW hit pyrE restoredThis study1105630 \Delta erm \Delta IID PMO_p630 \Delta erm \Delta IID AIIP AIM With pyrE restoredThis study1108630 \Delta erm \Delta IID PMO_p630 \Delta erm \Delta IID AIIP AIM With pyrE restoredThis study1111630 \Delta erm \Delta IID PMO_p630 \Delta erm \Delta IID AIIP AIM With pyrE restoredThis study1118630 \Delta erm \Delta IID PMO_p630 \Delta erm \Delta IID AIP AIM AV Hit pyrE restoredThis study1118$	907	$630\Delta erm\Delta pyrE \Delta IIM$	$630\Delta erm\Delta pyrE$ with spoIIM (CD1221) deleted	This study
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916 $630 \Delta erm \Delta IIQ$ -p $630 \Delta erm \Delta IIQ$ with pyrE restoredThis study919 $630 \Delta erm AIID Q$ -p $630 \Delta erm \Delta IIDAIIQ$ with pyrE restoredThis study922 $630 \Delta erm AIID AIQ$ $630 \Delta erm \Delta IIDAIIQ$ with pyrE restoredThis study946 $630 \Delta erm \Delta IIDAIIQ$ with ispL deletedThis study1001 $630 \Delta erm \Delta sipL$ -p $630 \Delta erm \Delta pyrE$ with sipL deletedThis study1010 $630 \Delta erm \Delta sipL$ -p $630 \Delta erm \Delta pyrE$ with sipL deletedThis study1011 $630 \Delta erm \Delta pyrE$ AIIDPMQ $630 \Delta erm \Delta pyrE$ with spDL D, spDIP, spDIQ, and spDIIM deletedThis study1018 $630 \Delta erm \Delta pyrE$ AIIDPP $630 \Delta erm \Delta pyrE$ with spDID, spDIP, spDIQ, and spDIIM deletedThis study1026 $630 \Delta erm \Delta pyrE$ AIIDPP $630 \Delta erm \Delta pyrE$ with spDID, spDIP, spDIM deletedThis study1037 $630 \Delta erm \Delta IIDPP-p$ $630 \Delta erm \Delta IIDAIP with pyrE restoredThis study1048630 \Delta erm AIIDP-p630 \Delta erm \Delta IIDAIP with pyrE restoredThis study1051630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDAIP With pyrE restoredThis study1111630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDAIPAW with pyrE restoredThis study1111630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDAIPAW with pyrE restoredThis study1118630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDPAIPA With pyrE restoredThis study1111630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDPAIPA With pyrE restoredThis study1111630 \Delta erm \Delta IIDPAP-p630 \Delta erm \Delta IIDPAP-PAPPAPPE sigE: erm B$	913	$630\Delta erm \Delta IIP$ -p	$630\Delta erm\Delta IIP$ with pyrE restored	This study
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1003 $630 \Delta erm \Delta pyr E \Delta sipL$ $630 \Delta erm \Delta pyr E$ with $sipL$ deletedThis study1010 $630 \Delta erm \Delta sipL-p$ $630 \Delta erm \Delta pyr E$ with $sipL$ deletedThis study1013 $630 \Delta erm \Delta sipL/sipL$ $630 \Delta erm \Delta pyr E$ with $sipL$ deletedThis study1014 $630 \Delta erm \Delta pyr E$ $\Delta IIDPMQ$ $630 \Delta erm \Delta pyr E$ with $spolID$ , $spolIP$ , $spolIQ$ , and $spolIM$ deletedThis study1054 $630 \Delta erm \Delta pyr E$ $\Delta IIDPM$ $630 \Delta erm \Delta pyr E$ with $spolID$ , $spolIP$ , and $spolIM$ deletedThis study1057 $630 \Delta erm \Delta IIDP-p$ $630 \Delta erm \Delta IIDP Apyr E$ with $spolID$ , $spolIP$ , and $spolIM$ deletedThis study105 $630 \Delta erm \Delta IIDPM-p$ $630 \Delta erm \Delta IIDAIIP AIIM with pyr E restoredThis study1105630 \Delta erm \Delta IIDPMQ-p630 \Delta erm \Delta IIDAIIP AIIM \Delta IIQ with pyr E restoredThis study1111630 \Delta erm \Delta IIDPMQ-p630 \Delta erm AIIDAIIP AIIM \Delta IIQ with pyr E restoredThis study1114630 \Delta erm A IIDPMQ-p630 \Delta erm AIIDAIIP AIIM \Delta IIQ with pyr E restoredThis study1114630 \Delta erm A IIDP MQ-p630 \Delta erm A IIDAIIP AIIM \Delta IIQ with pyr E locusThis study1114630 \Delta erm A IIDP MQ-p630 \Delta erm A IIDAIIP AIIM AIIQ with pyr E locusThis study1114630 \Delta erm A IIDP MQ-p630 \Delta erm A IIDAIIP AIIM AIIQ with pyr E locusThis study1155630 \Delta erm A IIDP MQ-p630 \Delta erm A IIDAIIP AIIM AIIQ with pyr E locusThis study1156630 \Delta erm A IIDQ IIDQ630 \Delta erm A IIDA PreP IOCUSThis study1263630 \Delta erm A IIDQ $	946	$630\Delta erm \Delta IIM/IIM$	$630\Delta erm\Delta IIM$ with IIM in the pvrE locus	This study
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1054630AermApyrE ΔIIDP630AermApyrE with spoIID and spoIIP deletedThis study1057630AermApyrE ΔIIDP630AermApyrE with spoIID, spoIIP, and spoIIM deletedThis study1102630Aerm ΔIIDP-p630AermAIIDAIIP with spoIID, spoIIP, and spoIIM deletedThis study1105630Aerm ΔIIDP-p630AermAIIDAIIP with pyrE restoredThis study1106630Aerm ΔIIDPM-p630AermAIIDAIIPAIIM with pyrE restoredThis study1108630Aerm ΔIIDPM-p630AermAIIDAIIPAIIM with pyrE restoredThis study1111630Aerm ΔIIDPM-p630AermAIIDAIIPAIIM with pyrE restoredThis study1111630AermAIIDAIP630AermAIIDAIIPAIIM with pyrE restoredThis study1111630AermAIIDPM-p630AermAIIDAIIPAIIMAIIQ with pyrE restoredThis study1111630AermAIIP-p630AermAIIDAIIPAIIMAIIQ with pyrE restoredThis study1111630AermAIIP-p630AermAIIDAIIQ630AermAIIDAIIQ20171115630AermAIIP-p630AermAIIDAIIQ with spoIID-spoIIQ in the pyrE locusThis study1126630AermAIIDP/mCherry-IVA630AermAIIDAIIQ with spoIID-spoIIQ in the pyrE locusThis study1263630AermAIIP/mCherry-IVA630AermAIIDAIIQ with cotE-mCherry in the pyrE locusThis study1306630AermAIIM/cotE-mCherry630AermAIIM with cotE-mCherry in the pyrE locusThis study1315630AermAIIM/cotE-mCherry630AermAIIM with cotE-mCherry in the pyrE locusThis study1316630AermAIIM/cotE-mCherry630AermAIIDAIIQ with spoIID_E101A in the pyrE locus	1018	$630\Lambda erm\Lambda mrE \Lambda IIDPMO$	$630\Lambda erm\Lambda nvrF$ with spoIID spoIIP spoIIO and spoIIM deleted	This study
1057630AermApyrE MIDPM630AermAppr With spoIID, spoIIP, and spoIM deleted1118 study1102630Aerm AIIDP-p630AermAIIDAIIP with pyrE restoredThis study1105630Aerm AIIDPM-p630AermAIIDAIIPAIM with pyrE restoredThis study1108630Aerm AIIDPM-p630AermAIIDAIIPAIM with pyrE restoredThis study1111630Aerm AIIDPM-p630AermAIIDAIIPAIMAVID with pyrE restoredThis study1111630AermAIIPA630AermAIIDAIIPAIMAVID with pyrE restoredThis study1111630Aerm/Cherry-IVA630AermAIIM with pyrE restoredThis study1112630Aerm/Cherry-IVA630AermApyrE sigK::ermBThis study1113630AermApyrE sigK630AermApyrE sigK::ermBThis study1114630AermAIID/IDD630AermAIIDAIIDAIIPAIMAVID with spoIID-spoIIQ in the pyrE locusThis study1115630AermAIID/IDD630AermAIIDAIID with spoIID-spoIIQ in the pyrE locusThis study11263630AermAIIP/mCherry-IVA630AermAIIDAIIW with cotE-mCherry in the pyrE locusThis study1306630AermAIIM/mCherry-IVA630AermAIID with cotE-mCherry in the pyrE locusThis study1319630AermAIIM/cotE-mCherry630AermAIIM with cotE-mCherry in the pyrE locusThis study1319630AermAIIM/cherry-IVA630AermAIIM with cotE-mCherry in the pyrE locusThis study1319630AermAIIM/cherry-IVA630AermAIIM with spoIID_enoIA in the pyrE locusThis study1444630AermAIIDPIIDE630AermAIIDAIIW with spoIID_enoIA in the pyrE locusThis study <t< td=""><td>1054</td><td><math>630\Lambda erm\Lambda mr E \Lambda IIDP</math></td><td><math>630\Lambda erm\Lambda nvrF</math> with spoIID and spoIIP deleted</td><td>This study</td></t<>	1054	$630\Lambda erm\Lambda mr E \Lambda IIDP$	$630\Lambda erm\Lambda nvrF$ with spoIID and spoIIP deleted	This study
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1105630Aerm AIIDPM-p630AermAIIDAIIPAIIP MW with pyrE restoredThis study1108630Aerm AIIDPMQ-p630AermAIIDAIIPAIIPAIIPAIIPAIIPAIIPAIIPAIIPAIIP	1102	$630\Lambda erm \Lambda IIDP$ -p	630 <i>AermAIIDAIIP</i> with <i>pvrE</i> restored	This study
1108630Aerm $\Delta IIDPMQ$ -p630Aerm $\Delta IIDPINIPMQ$ -p7111111111111111111111111111111111111	1105	$630 \Lambda erm \Lambda IIDPM-n$	630 <i>Aerm AIID AIIP MIM</i> with <i>pvrE</i> restored	This study
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1111Coolerm Link pCoolerm Link pCooler mCooler mCool	1111	$630\Lambda erm \Lambda IIM$ -p	630 <i>Aerm AIIM</i> with <i>pyrE</i> restored	This study
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1482 $630\Delta erm\Delta pyrE \Delta IIM\Delta sipL$ $630\Delta erm\Delta pyrE$ with $spoIIM$ and $sipL$ deletedThis study1485 $630\Delta erm\Delta pyrE$ $sigE^ 630\Delta erm\Delta pyrE$ $sigE:ermB$ This study1488 $630\Delta erm\Delta pyrE$ $sigF$ $630\Delta erm\Delta pyrE$ $sigF::ermB$ This study1520 $630\Delta erm\Delta IIP/sipL-mCherry$ $630\Delta erm\Delta IIP$ with $sipL-mCherry$ in the $pyrE$ locusThis study1522 $630\Delta erm\Delta IIM/sipL-mCherry$ $630\Delta erm\Delta IIM$ with $sipL-mCherry$ in the $pyrE$ locusThis study	1480	$630 \Lambda erm \Lambda myr E \Lambda IIP \Lambda sinL$	$630 \Lambda erm \Lambda nvrE$ with spoIIP and sinL deleted	This study
$1485$ $630\Delta erm\Delta pyrE sigE^ 630\Delta erm\Delta pyrE sigE:ermB$ This study $1488$ $630\Delta erm\Delta pyrE sigF^ 630\Delta erm\Delta pyrE sigF::ermB$ This study $1520$ $630\Delta erm\Delta IIP/sipL-mCherry$ $630\Delta erm\Delta IIP$ with $sipL-mCherry$ in the $pyrE$ locusThis study $1522$ $630\Delta erm\Delta IIM/sipL-mCherry$ $630\Delta erm\Delta IIM$ with $sipL-mCherry$ in the $pyrE$ locusThis study	1482	$630\Lambda erm\Lambda pyrE \Lambda IIM\Lambda sinI$	630 <i>AermApyrE</i> with <i>spoIIM</i> and <i>sipL</i> deleted	This study
1488 $630\Delta erm\Delta pyrE sigF$ $630\Delta erm\Delta pyrE sigF::ermB$ This study1520 $630\Delta erm\Delta IIP/sipL-mCherry$ $630\Delta erm\Delta IIP$ with $sipL-mCherry$ in the $pyrE$ locusThis study1522 $630\Delta erm\Delta IIM/sipL-mCherry$ $630\Delta erm\Delta IIM$ with $sipL-mCherry$ in the $pyrE$ locusThis study	1485	$630\Lambda erm\Lambda pvrE sigE^-$	$630\Lambda erm\Lambda nvrE sigE \cdots ermB$	This study
1520 $630\Delta erm\Delta IIP/sipL$ -mCherry $630\Delta erm\Delta IIP$ with $sipL$ -mCherry in the $pyrE$ locusThis study1522 $630\Delta erm\Delta IIM/sipL$ -mCherry $630\Delta erm\Delta IIM$ with $sipL$ -mCherry in the $pyrE$ locusThis study	1488	$630\Lambda erm\Lambda nvrE sigE$	$630\Lambda erm\Lambda nvrE sigF \cdot ermB$	This study
$1522$ $630\Delta erm\Delta IIM/sipL-mCherry$ $630\Delta erm\Delta IIM$ with $sipL-mCherry$ in the pyrE locus This study	1520	$630\Lambda erm\Lambda IIP/sinL-mCherry$	630 <i>Aerm</i> A <i>IIP</i> with <i>sinL</i> - <i>mCherrv</i> in the <i>pvrE</i> locus	This study
	1522	$630\Delta erm\Delta IIM/sipL-mCherrv$	$630\Delta erm\Delta IIM$ with <i>sipL-mCherry</i> in the <i>pyrE</i> locus	This study

1535	$630\Delta erm \Delta IIP/gpr-IIP_{E309A}$	$630\Delta erm\Delta IIP$ with gpr-spoIIP <sub>E309A</sub> in the pyrE locus	This study
1541	630∆ <i>erm sigF</i> <sup>−</sup> -p	$630 \Delta erm \ sigF$ :: ermB with pyrE locus restored	This study
1544	$630\Delta erm \ sigE^{-}-p$	$630 \Delta erm \ sigE::ermB$ with $pyrE$ locus restored	This study
1593	$630\Delta erm \ sigG^{-}p$	$630\Delta erm \ sigG::ermB$ with pyrE locus restored	This study
1643	$630\Delta erm \Delta IIO/IIO_{H120A}$	$630\Delta erm\Delta IIO$ with spoIIO <sub>H120A</sub> in the pyrE locus	This study
1649	$630\Delta erm\Delta pvrE IIP^-$	$630\Delta erm\Delta pvrE spoIIP::ermB$	This study
1672	$630 \wedge erm IIP^-$ -p	$630 \land erm \ spoIIP :: ermB \ with \ pvrE \ locus \ restored$	This study
1674	630 Aerm AIIDO/IIOH120A	$630 \Lambda erm \Lambda IID \Lambda IIO$ with spoIIO $\mu_{120\Lambda}$ in the pvrE locus	This study
1721	$630\Lambda erm \Lambda IIDO/IID$	$630 \Lambda erm \Lambda IID \Lambda IIO$ with spoIID in the pyrE locus	This study
1724	$630\Lambda erm \Lambda IIDO/IIO$	$630 \Lambda erm \Lambda IID \Lambda IIO$ with spoIIO in the pyrE locus	This study
1749	$630\Lambda erm \Lambda IIP/IIP_{E200\Lambda}$	$630 \Lambda erm \Lambda IIP$ with spoll $P_{r2004}$ in the pyrE locus	This study
2059	$630 \Lambda arm \Lambda mr F \Lambda IIPO$	630A arm Amer FAIIO with spoIIP deleted	This study
2055	$630 \Lambda arm \Lambda IIPO p$	630A arm AIIPO with the nurE locus restored	This study
2003	630A arm A IIPO/IIP	630A arm A IIPO with the pyrE locus restored	This study
2008	050 Zer m Zill Q/III E309A	booldermann g with sporn E309A in the pyre focus	This study
<i>E. coli</i> st	rains		
		$F-\Phi 80 lac Z\Delta M15 \Delta (lac ZYA-arg F) U169 recA1 endA1 hsdR17$	
41	DH5a	$(rK^{-}, mK^{+})$ phoA supE44 $\lambda$ - thi-1 gyrA96 relA1	D. Cameron
531	HB101/pRK24	F-mcrB mrr hsdS20(rB $\overline{mB}$ ) recA13 leuB6 ara-13 proA2 lavYI	C.
		galK2 xvl-6 mtl-1 rpsL20 carrying pRK24	Ellermeier
		Saure with a transfer out of the price of	(Fimlaid <i>et</i>
659	nIS107/sigE	nIS107-sigE targeting 119 hn in HB101/nRK24	$(1 \text{ minute } c_l)$ al = 2013)
057	piblomingE	piblo/ sight ungetting 119 op in 11D101/pick24	(Fimlaid <i>et</i>
681	nIS107/sigK	nIS107-sigK targeting 265 hp in HB101/nRK24	(1  minate  ei al. 2013)
001	p35107/31gK	pistor-sign targeting 205 op in HD101/pttt24	(Fimlaid <i>et</i>
735	nIS107/sigG	nIS107-sigG targeting 546 hp in HB101/nRK24	(1  minute  ei al. 2013)
155	p3510//3/g0	pistor-sigo targeting 540 op in HD101/pick24	(Fimlaid at
787	nIS107/sigE	nIS107 sigE torgeting 450 hp in HP101/nPK24	$(1^{11111111111111111111111111111111111$
1280	pJS107/Sigr	pJS107-Sigr targeting 459 op in HB101/pKK24	<i>u</i> ., 2013)
1265	$p_{JS107/III}$	pET21a grad $ID$ (deletion of N terminal 27 as) in $PI 21(DE2)$	This study
1300	$p \ge 121a - \Pi D_{\Delta 27aa}$	perzra-spond <sub><math>\Delta 27aa (deletion of N-terminal 27 ad) in BL21(DE3)</math></sub>	(Ng at al
1520	MTI VN12	MTL VN2 in DILL.	$(\log ei ai., 2012)$
1539	pm1L-YN3	pMTL-YN3 IN DH3a	2013) (Damaana at
1576	DGW1729	$T_{i}$	(Kansom <i>et</i>
15/6	pDSw1/28	I etracycline-inducible <i>mCherry</i>	<i>al.</i> , 2015)
1630	$pM1L-YN3-\Delta IIP$	pMTL-YN3- <i>AspoIIP</i> in HB101/pKK24	This study
1641	$pM1L-YN3-\Delta IIQ$	$pM1L-YN3-\Delta spo11Q$ in HB101/pRK24	This study
1642	$pMIL-YN3-\Delta IID$	pM1L-YN3-AspoIID in HB101/pRK24	This study
1.660			(Ng et al.,
1662	pMIL-YNIC	pM1L-YNIC in HB101/pRK24	2013)
1670	$pMTL-YN3-\Delta IIDQ$	pMTL-YN3- <i>AspoIID-spoIIQ</i> in HB101/pRK24	This study
1675	$pMTL-YN3-\Delta IIM$	pMTL-YN3-Δ <i>spoIIM</i> in HB101/pRK24	This study
1676	pMTL-YN1C- <i>IIQ</i>	pMTL-YN1C- <i>spoIIQ</i> in HB101/pRK24	This study
1677	pMTL-YN1C-IID	pMTL-YN1C-spoIID in HB101/pRK24	This study
1704	pMTL-YN3- <i>sipL</i>	pMTL-YN3- <i>ΔsipL</i> in HB101/pRK24	
1711	pMTL-YN1C-IIM	pMTL-YN1C- <i>spoIIM</i> in HB101/pRK24	This study
1718	pMTL-YN1C-sipL	pMTL-YN1C-sipL in HB101/pRK24	This study
1768	pMTL-YN1C-mCherry-IVA	pMTL-YN1C-mCherry spoIVA in HB101/pRK24	This study
1777	pMTL-YN1C-sipL-mCherry	pMTL-YN1C-sipL-mCherry in HB101/pRK24	This study
1789	pMTL-YN1C-IIDQ	pMTL-YN1C- <i>spoIID-spoIIQ</i> in HB101/pRK24	This study
1812	pMTL-YN1C- <i>cotE</i> -mCherry	pMTL-YN1C-cotE-mCherry in HB101/pRK24	This study
1845	pET28a- <i>IIP</i> $_{\Delta 27aa}$	pET21a- <i>spoIIP</i> $_{\Delta 27aa}$ (deletion of N-terminal 27 aa) in BL21(DE3)	This study
1901	pMTL-YN1C-IID <sub>E101A</sub>	pMTL-YN1C-spoIID <sub>E101A</sub> in HB101/pRK24	This study
1902	pMTL-YN1C-gpr-IIP <sub>E309A</sub>	pMTL-YN1C-gpr-spoIIP <sub>E309A</sub> in HB101/pRK24	This study
1967	pMTL-YN1C-IIQ <sub>H120A</sub>	pMTL-YN1C-spoIIQ <sub>H120A</sub> in HB101/pRK24	This study
1997	pMTL-YN1C-IIP <sub>F309A</sub>	pMTL-YN1C-spoIIP <sub>E309A</sub> in HB101/pRK24	This study
		- · · · · · · ·	-

# Supplementary Table S2. Primers used in this study.

Primer	Name	Sequence
532	3' Universal EBS	CGAAATTAGAAACTTGCGTTCAGTAAAC
1177	5' NotI <i>spoIIQ</i> ( <i>CD0125</i> )	AGAATGCGGCCGCCCACATTACTACACAGATAC
1178	3' XhoI spoIIQ	AATA <u>CTCGAG</u> GTTATACTATTACTTAATTAGACTC
1487	5' IBS1 spoIIP 798 (CD2469)	AAAAAAGCTTATAATTATCCTTACTAAGCTGTTTTGTGCGCCCAGATAGGGTG
1488	3' EBS1d spoIIP 798	CAGATTGTACAAATGTGGTGATAACAGATAAGTCTGTTTTCTTAACTTACCTTTCTTGT
1489	5' EBS2 <i>spoIIP</i> 798	TGAACGCAAGTTTCTAATTTCGATTCTTAGTCGATAGAGGAAAGTGTCT
1558	5' spoIID (42) (CD0126)	GCTCTGTGTTAGTTCCATCAC
1560	5' <i>spoIIP</i> (124)	GAAGCTAAAGTCGAAGGAAAT
1561	3' XhoI spoIIP no stop (1018)	ATTA <u>CTCGAG</u> ATTTTTTGTTTAAAATATTC
1563	5' CD1221 (61) spoIIM	GGATTGTTGTTTATGATGTCTATT
1565	3' CD1221 (532) spoIIM	CAACCCCAATGATAGTGCTATTA
1572	5' NotI <i>spoIID</i>	AAAA <u>GCGGCCGC</u> GCTACTGATTTAAGAGCAGG
1573	3' XhoI spoIID	ATTA <u>CTCGAG</u> GATTATTGACGTGTTTTTGG
1576	5' NotI spoIIM (CD1221)	AAAA <u>GCGGCCGC</u> CAACAGGTCTTACTCCAGG
1577	3' XhoI spoIIM	ACAAG <u>CTCGAG</u> GATGCCACTGTATTCTCTG
1631	5' NheI His <sub>6</sub> -SpoIID $_{\Delta 27aa}$	AAA <u>GCTAGC</u> ATGCATCATCATCATCATAAAAATGTAGAATTAACTG
1632	3' XhoI spoIID with stop	A <u>CTCGAG</u> TTAGTATATATCTTTTATTTTTG
1658	3' <i>spoIID</i> (367) for qRT	CTACATATGTTCTAGCTGCTAC
1759	5' Δ <i>sipL</i> SOE ( <i>CD3567</i> )	GATGTAATTAAAGTTGACAATAGAATAGACCCTGGGAAGTGTCTTATTTTAG
1760	$3' \Delta sipL$ rev oes	CTAAAATAAGACACTTCCCAGGGTCTATTCTATTGTCAACTTTAATTACATC
1850	5' <i>spoIIQ</i> H120A SOE	TCTAAAACACTTGATGTTTGGGAAACTGCTAAAGGTGTAGATATTAGTTGTACTAAAG
1851	3' spoIIQ H120A rev oes	CTTTAGTACAACTAATATCTACACCTTTAGCAGTTTCCCAAACATCAAGTGTTTTAGA
1941	5' AscI ΔspoIIP	AAT <u>GGCGCGCC</u> TTAGCATTTAATTTATACTGGAGGTTTATATG
1942	5' $\Delta spoIIP$ SOE	GACTTTTATATTAGTATGCATTTTACCAGGAGCCGAGATTTTGGATGAATATTTTAAAC
1943	3' $\Delta spoIIP$ rev eos	GTTTAAAATATTCATCCAAAATCTCGGCTCCTGGTAAAATGCATACTAATATAAAAGTC
1944	3' SbfI Δ <i>spoIIP</i>	ATT <u>CCTGCAGG</u> TCCTCTGGTCTTGCACTTG
1945	5' Δ <i>spoIIP</i> flanking	CTGACAAATCACTGACTAAACCC
1946	3' Δ <i>spoIIP</i> flanking	CTTCATTATCTCCTTTAGGTGG
1959	5' AscI ΔspoIID	TTAA <u>GGCGCGCC</u> GTGCAAAGATAGAAATGGACCATGG
1960	5' ΔspoIID SOE	CCATTGGTTGTTTGTTAGGATTTGTGGAGGGCTATAAATATTATGATATATTATCAC
1961	3' Δ <i>spoIID</i> rev eos	GTGATAATATATCATAATATTTATAGCCCTCCACAAATCCTAACAAAACAACCAATGG
1962	3' SbfI Δ <i>spoIID</i>	CTA <u>CCTGCAGG</u> CGGTTTGTCTTACTGTAGTG
1963	5' Δ <i>spoIID</i> flanking	GCCAGGAGGATGTGCTATAG
1964	3' Δ <i>spoIID</i> flanking	CCTTCTCATATTTTAATTTCGTTGCTAATCC
1965	5' AscI ΔspoIIQ	TTA <u>GGCGCGCC</u> CAGAGAGCCCAGTATCTATAAAC
1967	5' $\Delta spoIIQ$ SOE	GTTTGTTTATTAGCAGTAGGTGGAGTTTGGGGTATACATGTGCATTTAGAGGCG
1968	$3' \Delta spoIIQ$ rev eos	CGCCTCTAAATGCACATGTATACCCCAAACTCCACCTACTGCTAATAAACAAAC
1969	3' SbfI ΔspoIIQ	TTG <u>CCTGCAGG</u> CTATGGCAGCTGCTATTGGTTC
1970	5' Δ <i>spoIIQ</i> flanking	GGTGGTCAAATCGAAATAGTAGAAG
1971	3' Δ <i>spoIIQ</i> flanking	CTCTGCTGTTCTTCTCCTATAAG
1973	5' Δ <i>spoIIDQ</i> SOE	GGATTTGTGACTTGCTCTGTGGCGTATAGTGGAGAAAAATCTATAGAC
1974	$3' \Delta spoIIDQ$ rev eos	GTCTATAGATTTTTCTCCACTATACGCCACAGAGCAAGTCACAAATCC
1995	5' AscI ∆sipL Gibson	gtcaattgttcaaaaaaataatggc <u>GGCGCGCC</u> GCTCAACTTGGAATACCTGC
1997	5' ∆ <i>spoIIM</i> flanking	GATGCAGTATTTGTCAGTATG
1998	3' ∆ <i>spoIIM</i> flanking	CTATACCATCTATGTTTC
1999	5' AscI ΔspoIIM	gtcaattgttcaaaaaaataatggcGGCGCCGATTGAACAAGGCTCTATTC
2000	5' $\Delta spoIIM$ SOE	agcaaggcaagaccgatcgggcccCCTGCAGGGTACTTACATCCTTACTATCAC

2001	3' $\Delta spoIIM$ rev eos	TAGACAAATAATTATTATAGGATTGGCAAGTATTGGTATTATTAAGTTCCTAG
2002	3' SbfI ∆ <i>spoIIM</i>	CTAGGAACTTAATAATACCAATACTTGCCAATCCTATAATAATTATTTGTCTA
2088	3' SbfI $\Delta sipL$ gibson	agcaaggcaagaccgatcgggccccc <u>ctgcag</u> gGTTTGTTAGCTTTTCTCCAATACCTTG
2133	3' XhoI mCherry Gibson	gccaagcttgcatgtctgcaggc <u>ctcgag</u> TTATTTATATAATTCATCCATACCTCCTGTTG
2138	3' spoIIM internal colony PCR	GCAACCCCAATGATAGTGC
2165	5' NotI sipL Gibson	ggaattagggatgtaataagcggccgcGAGAGACATATAGGAAAGAAAATATTG
2195	3' spoIID internal to deletion	GACCTTTAGTTCCTCTAACTGCTTC
2196	3' <i>spoIIQ</i> internal to deletion	CACCTTTATGAGTTTCCCAAACATC
2197	3' spoIIP internal to deletion	CCATCCTTTGCTATCTAAAGCACTAG
2211	5' SipL-mCherry linker SOE	CTTATTTTAGAAAAAAAGTCGTATTAGTAGATGCAATGGTATCTAAAGGAGAAGAAGAAGA
2212	3' SipL-mCherry linker rev	ATCTTCTTCTCCTTTAGATACCATTGCATCTACTAATACGACTTTTTTTT
2231	5' NotI spoIID Gibson	ggaattagggatgtaataagcggccgcCGCTACTGATTTAAGAGCAGG
2232	3' XhoI spoIIQ Gibson	caagettgcatgtctgcaggc <u>ctcgag</u> GTTATACTATTACTTAATTAGACTC
	5' CotE-mCherry SOE	
2263	(CD1433)	CTTGAAGGTATTTATGGGCAATTCGCAATGGTATCTAAAGGAGAAGAAGAT
2264	3' CotE-mCherry rev eos	ATCTTCTTCTCCTTTAGATACCATTGCGAATTGCCCATAAATACCTTCAAG
2268	5' NotI cotE Gibson	ggaattagggatgtaataagcggccgcTTCAATCCACTCACCTCGTATAATC
	5' NcoI <i>spoIIP</i> pET28a	
2297	Gibson	GTTTAACTTTAAGAAGGAGATATACCATGGCACAAGATGATTTTTTAAAGTTTTTAG
	3' XhoI <i>spoIIP</i> pET28a	
2316	Gibson	
2352	5' NotI gpr Gibson	ggaattagggatgtaataagcggccgcCCTATATAAATAGTTGCTACCTTTTTCTG
2378	3' XhoI spoIID YN1C Gibson	caagettgcatgtctgcaggc <u>ctcgag</u> GATTATTGACGTGTTTTTGGCATTTTATAC
2379	5' spoIID E101A SOE	CTATTTATGTGGAGTACTAGCTGGTGCGATGTCTTCAGAGTTTGATATAGAAGC
2380	3' spoIID E101A rev eos	GCTTCTATATCAAACTCTGAAGACATCGCACCAGCTAGTACTCCACATAAATAG
2381	5' spoIIP E309A SOE	GTTTGCAGTTAAGAATCATATGTTAGTAGCAATAGGGAATAACGCAACAAGTGTAG
2382	3' <i>spoIIP</i> E309 rev eos	CTACACTTGTTGCGTTATTCCCTATTGCTACTAACATATGATTCTTAACTGCAAAC
	3' XhoI CD2468 YN1C	
2383	Gibson	caagcttgcatgtctgcaggc <u>ctcgag</u> CTACTTATTAAATTTTTTTATGATTTCATG
2449	5' NotI spoIIQ Gibson	ggaattagggatgtaataagcggccgcCACATTACTACACAGATACAAAAATAAAAG
2481	5' NotI spoIIP 269 gibson	ggaattagggatgtaataagcggccgcACTCTTACAATAGATGTCTTAGATATGGC

Restriction sites are underlined.

**Supplementary Table S3 -** Sporulation heat resistance ratios determined for all strains across all replicates.

This table can be accessed using the following link:

https://www.dropbox.com/s/ew6lylqvovbl6qp/Supplementary%20Table%20S3.xlsx?dl=0

Heat resistance (H.R.) efficiencies were determined from 23 hr cultures and represent the mean and standard deviation for a given strain relative to wild type based on a minimum of three independent biological replicates. The limit of detection of the assay is  $10^{-6}$ .

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