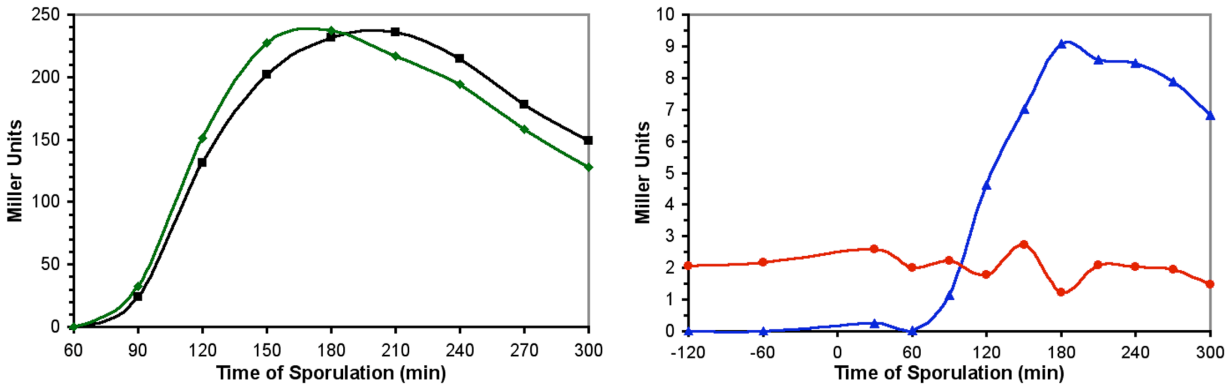
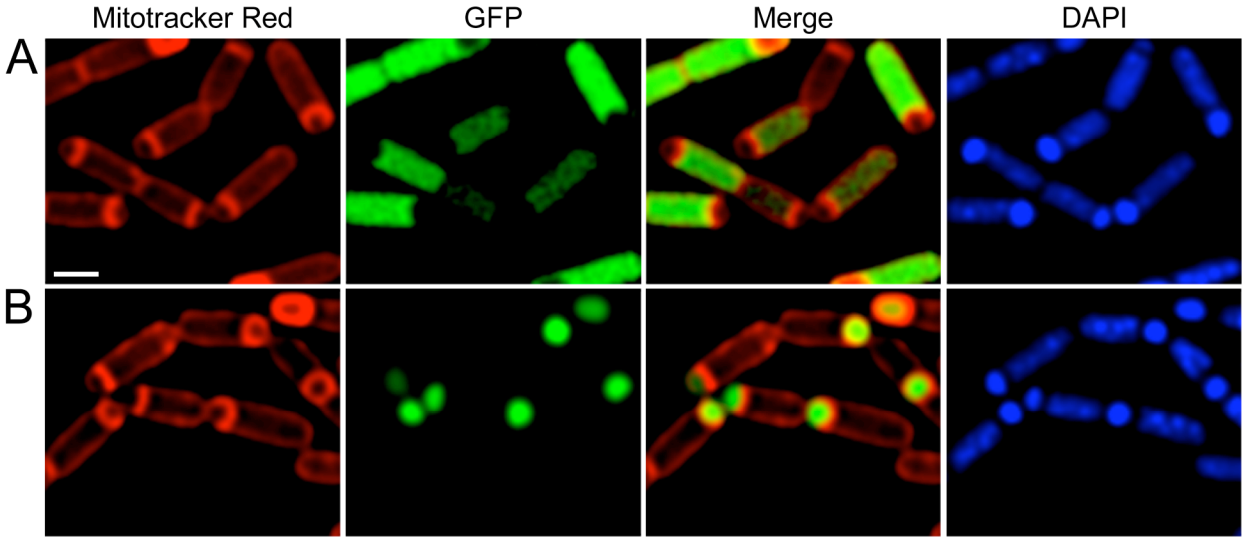
**Supplemental Information**

**Supplemental Figure 1: β-galactosidase Activity**. To assess the activity of the various promoters used in this study, we constructed translational *lacZ* fusions as described below, and integrated these plasmids onto the chromosome, resulting in strains KP642 (*amyE::*$P_{spoIIIE}$*-lacZ-cat*), KP643 (*amyE::*$P_{spoIIQ}$*-lacZ-cat*), KP644 (*amyE::*$P_{spoIID}$*-lacZ-cat*), and KP645 (*amyE::*$P_{spoIIR}$*-lacZ-cat*). Samples were taken throughout sporulation and during early and late logarithmic growth for KP642, and β-galactosidase assays performed as described (1). The low level of background β-galactosidase activity from strain PY79 was subtracted, and the results graphed. Red circles represent KP642, black squares KP643, green diamonds KP644, and blue triangles KP645.



**Supplemental Figure 2: Localization of GFP produced under the *spoIID* and *spoIIQ* promoters.** The figure shows localization of GFP (green) produced by either the mother cell-specific *spoIID* (A) or the forespore-specific *spoIIQ* (B) promoters. In both cases, GFP fills each cell and appears entirely cytoplasmic. Samples were taken two hours after the initiation of sporulation, stained with Mitotracker Red (red) and DAPI (blue) and visualized as described in the main text.



**Supplemental Methods: Plasmid and Strain Construction**

All plasmids are derivative of pDG1662 (2), all cloning was done in *Escherichia coli* DH5, restriction enzymes and T4 DNA ligase were obtained from New England Biolabs. PCR was done using Expand High Fidelity Polymerase (Roche). We began by constructing pMDS12, a plasmid containing a promoterless copy of superbright GFP mutant 1 (3). The GFP gene was amplified using the primers GACTGAGAATTCGGATCCAAGCTTACTAGT**AGTAAAGGAGAAGAACTTTTCAC TGG** and CTGACTAGATCT**CTATTACGGCCGTTTGTATAGTTCATCCATGCCAT-GTG** (coding sequence is shown in bold) and cleaved with EcoRI and BglII. This digested fragment was then ligated into pDG1662 (2) digested with EcoRI and BamHI. The plasmids pMDS13, pMDS14, pMDS16 and pMDS78 contain the *spoIIQ*, *spoIID*, *spoIIIE* and *spoIIR* promoters, respectively translationally fused to the *gfp* gene of pMDS12. A ~500 bp *spoIIQ* promoter fragment was amplified with the primers GACTGAAGATCTGCTAGC**GCCATAAGTGAGCGGATGCCAAG** and CTGACTA-AGCTTGGATCC**GTTTTCTTCCTCTCTCATTGTTTCATC**, cleaved with BglII and HindIII and ligated into BamHI and HindIII digested pMDS12. The *spoIID* promoter fragment, containing roughly 900 base pairs of DNA upstream of the translational initiation codon, was amplified using the primers GACTGAAGATCTGCTAGC**GTTGATTTAGCAAACTATATCAACGG** and CTGA-TCACTAGTGGATCC**TGCGAATTGTTTCATATTCAGCTGC**, digested with BglII and SpeI and ligated into BamHI and SpeI digested pMDS12. The *spoIIIE* promoter fragment, containing approximately 1 kb of DNA upstream of the *spoIIIE* translational initiation site, was amplified with the primers GACTGAAGATC-TGCTAGC**AACGTAAACCGATGATCATCC** and CTGACTAAGCTTGGATCC**TT-TCTTTGCCACACTCATCACCTTAC,** digested with BamHI and HindIII, and cloned

into BamHI and HindIII digested pMDS12. Although no specific consensus promoter sequences could be identified in this region, the fragment appears to contain a very low activity promoter which is capable, when fused to the *spoIIIE* structural gene, of fully complementing either a *spoIIIE* null or point mutant (data not shown). Finally, the *spoIIR* promoter fragment was amplified with the primers GACTAGATCT**GCTTTCTTTGTTGCGGCCATACC** and CTGAAAGCTTGGATCC-**TACTGTTTTTTTCATCGGTCCCCAC**, digested with BglII and HindIII, and cloned into BamHI and HindIII digested pMDS12. All four plasmids were integrated onto the *B. subtilis* chromosome via a double recombination at the *amyE* locus (described below) and tested for GFP production.

After confirming these clones expressed GFP, we constructed pMDS24, pMDS27, pMDS33, and pMDS80 which contain the full length *spoIIIE* gene inserted between the promoters and *gfp*. First, we amplified the *spoIIIE* coding region with the primers CTGACTGGATCC**CGAAAATCAAGAAAAAAACAGGCGAAA** and GACTGAACT-AGT**AGAAGAGACCTCATCATATTTCTCTT**, digested it with BamHI and SpeI, then ligated it into pMDS14 digested with BamHI and SpeI yielding pMDS27. After confirming the gene sequence was correct and functional in *B. subtilis* we subcloned the BamHI-SpeI fragment from pMDS27 into pMDS13, pMDS16, and pMDS78 to produce pMDS24, pMDS33, and pMDS80 respectively. Construction of the truncated versions of *spoIIIE* was accomplished in a similar fashion. For the membrane domain, a PCR fragment containing the coding sequence for the first 183 amino acids of SpoIIIE was amplified with the primers CTGACTGGATCC**CGAAAATCAAGAAAAAAACAGGC-GAAA** and GACTGAACTAGT**CCACTTTTTTAGCGTTTCTTGCAGC**, digested with BamHI and SpeI and ligated into pMDS13, pMDS14, pMDS16, and pMDS78 digested with the same enzymes. For the cytoplasmic domain, a fragment containing the coding

sequence for the last 609 amino acids amplified with the primers CTGACTACTAGT**CGCTCGCTGAAGAAACGC** and GACTGAACTAGT**AGAAG-AGAGTCTATCATATTTCTCTT**, digested with SpeI and ligated into each vector digested with SpeI. Orientation of the insert was confirmed by PCR screening.

In parallel we also constructed *lacZ* fusions to the *spoIIQ*, *spoIID*, *spoIIIE*, and *spoIIR* promoters. To accomplish this we replaced the *gfp* gene in each of pMDS13, pMDS14, pMDS16, and pMDS78 with the *lacZ* coding sequence from *E. coli* MC4100 produced with the primers GACTGGATCCACTAGT**ACCATGATTACGGATTC-ACTG** and CTGAAAGCTTCGGCCG**TTATTATTTTTGACACCAGACCAAC**. The *gfp* gene was released by digesting each plasmid with SpeI and EagI, and replaced with the PCR fragment containing the *lacZ* gene digested with the same enzymes. Activity of the new *lacZ* fusions was first confirmed in *E. coli* by assaying β-galactosidase activity on plates containing X-gal and later in *B. subtilis* with a β-galactosidase activity assay as described in supplemental Figure 1. Once each plasmid was constructed it was integrated by a double recombination event into the *B. subtilis* chromosome at the *amyE* gene (2).

**Supplemental Table 1: List of strains.**

| Strain | Genotype[1] |
|---|---|
| PY79 | wild type |
| KP141 | Δ*spoIIIE::spc* |
| KP92 | *spoIIIE36* |
| KP629 | *amyE::P*$_{spoIIIE}$*-spoIIIE-gfp-cat, spoIIIE::spc* |
| KP630 | *amyE::P*$_{spoIIQ}$*-spoIIIE-gfp-cat, spoIIIE::spc* |
| KP631 | *amyE::P*$_{spoIID}$*-spoIIIE-gfp-cat, spoIIIE::spc* |
| KP632 | *amyE::P*$_{spoIIR}$*-spoIIIE-gfp-cat, spoIIIE::spc* |
| KP633 | *amyE::P*$_{spoIIIE}$*-spoIIIE-gfp-cat, spoIIIE36* |
| KP634 | *amyE::P*$_{spoIIQ}$*-spoIIIE-gfp-cat, spoIIIE36* |
| KP635 | *amyE::P*$_{spoIID}$*-spoIIIE-gfp-cat, spoIIIE36* |
| KP636 | *amyE::P*$_{spoIIR}$*-spoIIIE-gfp-cat, spoIIIE36* |
| KP637 | *amyE::P*$_{spoIIIE}$*-spoIIIE*$_{mss}$*-gfp-cat, spoIIIE::spc* |
| KP638 | *amyE::P*$_{spoIIQ}$*-spoIIIE*$_{mss}$*-gfp-cat, spoIIIE::spc* |
| KP639 | *amyE::P*$_{spoIID}$*-spoIIIE*$_{mss}$*-gfp-cat, spoIIIE::spc* |
| KP640 | *amyE::P*$_{spoIIQ}$*-spoIIIE*$_{cyto}$*-gfp-cat* |
| KP641 | *amyE::P*$_{spoIID}$*-spoIIIE*$_{cyto}$*-gfp-cat* |
| KP642 | *amyE::P*$_{spoIIIE}$*-lacZ-cat* |
| KP643 | *amyE::P*$_{spoIIQ}$*-lacZ-cat* |
| KP644 | *amyE::P*$_{spoIID}$*-lacZ-cat* |
| KP645 | *amyE::P*$_{spoIIR}$*-lacZ-cat* |
| KP646 | *amyE::P*$_{spoIIQ}$*-gfp-cat* |
| KP647 | *amyE::P*$_{spoIID}$*-gfp-cat* |

[1]All strains are derivatives of PY79

**Supplemental Table 2: List of Plasmids**

| Plasmid | Insert |
|---|---|
| pMDS12 | *amyE::gfp cat* |
| pMDS16 | *amyE::$P_{spoIIIE}$-gfp cat* |
| pMDS13 | *amyE::$P_{spoIIQ}$-gfp cat* |
| pMDS14 | *amyE::$P_{spoIID}$-gfp cat* |
| pMDS78 | *amyE::$P_{spoIIR}$-gfp cat* |
| pMDS33 | *amyE::$P_{spoIIIE}$-spoIIIE-gfp cat* |
| pMDS24 | *amyE::$P_{spoIIQ}$-spoIIIE-gfp cat* |
| pMDS27 | *amyE::$P_{spoIID}$-spoIIIE-gfp cat* |
| pMDS80 | *amyE::$P_{spoIIR}$-spoIIIE-gfp cat* |
| pMDS42 | *amyE::$P_{spoIIIE}$-spoIIIE$_{mss}$-gfp cat* |
| pMDS40 | *amyE::$P_{spoIIQ}$-spoIIIE$_{mss}$-gfp cat* |
| pMDS41 | *amyE::$P_{spoIID}$-spoIIIE$_{mss}$-gfp cat* |
| pMDS34 | *amyE::$P_{spoIIQ}$-spoIIIE$_{cyto}$-gfp cat* |
| pMDS35 | *amyE::$P_{spoIID}$-spoIIIE$_{cyto}$-gfp cat* |
| pMDS74 | *amyE::$P_{spoIIIE}$-lacZ cat* |
| pMDS72 | *amyE::$P_{spoIIQ}$-lacZ cat* |
| pMDS73 | *amyE::$P_{spoIID}$-lacZ cat* |
| pMDS81 | *amyE::$P_{spoIIR}$-lacZ cat* |

**References**


1. J. Miller, in *Experiments in Molecular Genetics* . (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1972) pp. 352-355.
2. A. M. Guerout-Fleury, N. Fransdsen, P. Stragier, *Gene* **180**, 57-61 (1996).
3. B. P. Cormack, R. H. Valdivia, S. Falkow, *Gene* **173**, 33-38 (1996).