Supplementary data

Construction of the soj-yyaC and yyaA-yyaC deletion strains

To delete the soj-yyaC region from the B. subtilis chromosome, DNA fragments from upstream of the soj gene (yyaA to yyaB, 1510 bp) and downstream of the yyaC gene (N-terminal coding part of yyaD, 1930 bp) were amplified from the wild type strain SG38 by PCR, digested with BamHI and EcoRI, then ligated to the spectinomycin resistance gene excised from pIC156 (with BamHI and EcoRI). The ligation mixture was transformed into the wild type B. subtilis strain SG38 directly, with selection for spectinomycin resistance. One of the transformants was examined by PCR to confirm the deletion of soj-spo0J-yyaC, and the strain was designated 1223.

The yyaA-yyaB-soj-spo0J-yyaC region was deleted from the B. subtilis chromosome using the same method. The antibiotic resistance markers used were kanamycin from pSG122 and erythromycin from pSG840, and the strains obtained were 1221 and 1222, respectively.

Integration of the neo'-spc cassette and the 'neo-tet cassette on the B. subtilis chromosome

To allow chromosome inversion at the fixed points, in the ycgA gene and in the intergenic region between ydgH and ydgI, a cassette containing an intact spectinomycin resistance gene and a neomycin resistance gene truncated at its 3’ end (neo'-spc) was excised from plasmid pSG4914 (digested with SstI and SalI) and introduced into the wild type B. subtilis strain 168 using the method described above (with selection for spectinomycin resistance). Insertions within the ycgA gene (the strain was designated 1258) and at the intragenic region between ydgH and ydgI (strain 1259) were confirmed by long range PCR.

The same method was also used to insert the 'neo-tet cassette (from pSG4906 digested with SstI and SalI) at 12 different locations on the left arm of the chromosome. The transformants were selected for tetracycline resistance and the insertions were all confirmed by long range PCR.

Construction of the chromosome inversion strains

To construct the inversion strains (in the spoIIIE36 Δ(yyaA-yyaC)::ermC background unless otherwise stated), strains containing the 'neo-tet cassette at various locations on the chromosome were transformed with the chromosomal DNA from strain 1258 (containing the neo'-spc cassette at ycgA) or 1259 (containing the neo'-spc cassette between ydgH and ydgI). The transformants were streaked on plates containing kanamycin, and two of the kanamycin-resistant colonies, produced by recombination between the homologous region of the truncated neo genes
in the two cassettes (Figure 2A), were re-streaked on kanamycin selection plates at least 3 times. Some of the recombinants were examined by long range PCR to confirm the recombination.

**Construction of strains containing the lacO / gfp-lacI system at the dinB (+608) locus**

To allow the integration of the lacOx256 array at the dinB locus, a 0.8 kb DNA fragment spanning the end of the dinB gene and the beginning of the downstream ydgF gene was amplified from the wild type strain SG38 using PFU DNA polymerase and primers ydgF-F and dinB-R. The PCR product was digested with HindIII and cloned into pAT12 at the HindIII site to create pSG4918. Then the 2.3 kb DNA fragment containing Pxyl-gfp-lacI was amplified from pSG1189 using primers Spec3-F(SstI) and amyE-5’-F(PstI), and digested with SstI and XhoI before being cloned into pSG4918 (digested with the same enzymes). This generated pSG4919. Since the Pxyl promoter is down-regulated during sporulation, to improve the GFP signal the Pxyl promoter in pSG4919 was replaced with a σH-dependent spoVG promoter (amplified from SG38 using primers spoVG-F(SstI) and spoVG-R(KpnI)) using the SstI and KpnI sites. The resultant plasmid pSG4920 was then transformed into a strain containing cassettes for inversion from –214 to +612, or from –315 to +612, to generate strains 1273 and 1275, respectively.