Supplementary Materials

We tested our hypothetic strategy on 473 promoter sequences from *E. coli* [Hersberg *et al.* (2001), *Nucleic Acids Res.*, **29**, 277] which include 44 supercoiling-sensitive promoters [Peter *et al.* (2004), *Genome Biol.*, **5**, R87]. Most bacterial promoters contain two consensus sequences that are essential for binding RNA polymerase. One resides at ~10-bp upstream of the transcription start site (+1) and has the consensus sequence 5' TATAAT 3', and the other is centered ~35-bp upstream and has the consensus sequence 5' TTGACA 3'. Because the spacer between the hexameric motifs at or near -35 and -10 positions is important for transcription efficiency [Jordi *et al.* (1995), *EMBO J.*, **14**, 5690-5700], we compared the frequencies of all possible trinucleotides between the -35 and -10 regions with those between the -75 and +25 positions. AAA, GCG, ACG, and CCA have low bending flexibilities [Brukner *et al.* (1995), *EMBO J.*, **14**, 1812-1818] and appeared less frequently between the -35 and -10 regions of promoters insensitive to changes in superhelicity than they did in supercoiling-sensitive promoters (Fig. S1). The lower flexibility of the spacer in supercoiling-sensitive promoters, relative to supercoiling-insensitive promoters, may inhibit efficient interaction with RNA polymerase when the DNA is in the relaxed state; the flexibility of this spacer region might then be enhanced by supercoiling, as has been suggested by experiments performed on various mutated forms of the *proU* promoter [Jordi *et al.* (1995), *EMBO J.*, **14**, 5690-5700].

However, the frequencies of the appearance of the CAG/CTG trinucleotides were nearly the same for the spacer as they were for the -75 to +25 region, regardless of
the promoter’s sensitivity to changes in superhelicity (Fig. S1). Therefore, bacterial promoters do not appear to adopt the CAG/CTG triplet for responding to changes in superhelicity at least within our knowledge.

Fig. S1 Comparison of trinucleotide frequencies in *E. coli* promoter regions For the supercoiling-sensitive (open circle) and supercoiling-insensitive (filled circle) promoters, frequency of all trinucleotides was calculated in two regions: a spacer region between the -35 and -10 consensus sites and the control region at between -75 and +25 position. For the given trinucleotide, frequencies in the two regions are compared by plotting a point at a position determined by the control (horizontal-axis) and spacer region (vertical-axis) frequencies of that sequence element. Trinucleotides that are used more often at the spacer region will be plotted above the diagonal.