Gene syntax defines supercoiling-mediated transcriptional feedback

Gene syntax—the order and arrangement of genes and their regulatory elements—shapes the dynamic coordination of both natural and synthetic gene circuits. Transcription at one locus profoundly impacts the transcription of nearby adjacent genes, but the molecular basis of this effect remains poorly understood. Here, using integrated reporter circuits and chromatin conformation and sequencing techniques in human cells, we show that supercoiling-mediated feedback regulates expression of adjacent genes in a syntax-specific manner. Using syntax as a design parameter, we built compact gene circuits, tuning the mean, variance, and stoichiometries of expression across diverse delivery methods and cell types. Integrating supercoiling-mediated feedback into models of gene regulation will expand our understanding of native systems and enhance the design of synthetic gene circuits.