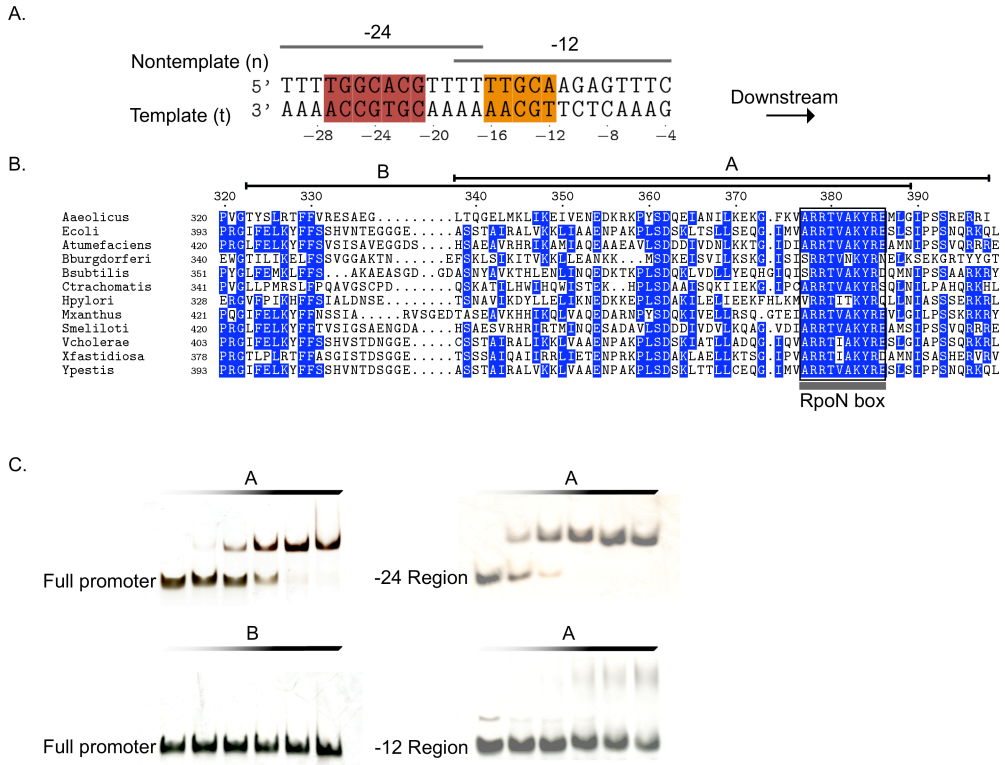
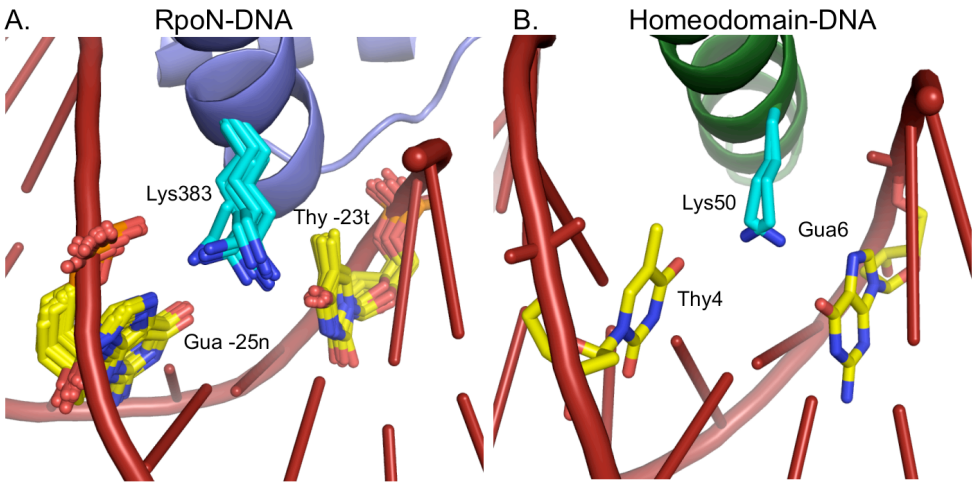
Supplemental Figure 1



Supplemental Figure 1: The last nine residues of σ^{54} are required for DNA affinity. (A)Full σ^{54} promoter element found upstream of the *NirB* gene in *Aquifex aeolicus*; the -24 and -12 consensus sequences are in red and orange, respectively. (B)Protein sequence alignment of the C-terminal end of σ^{54}. Residues conserved in ≥50% of the sequences are highlighted in blue. Protein constructs ("A," "B") that were used to determine the minimal DNA-binding region are shown above the sequence, and the signature RpoN motif is boxed. (C)Gel mobility assays of the RpoNdomain constructs binding to the σ^{54} promoter. *Left*: Construct "A" (top) binds to the full σ^{54} promoter sequence, but construct "B" (bottom) has no detectable binding under these assay conditions. *Right*: Construct "A" binds to the -24 element (top) but binds only non-specifically to the -12 element (bottom). The oligonucleotides used in this assay are shown above the sequence in (A); DNA concentrations were 40 and 70 μM with final protein concentrations 210 and 250 μM for the assays on the left and on the right, respectively.

Supplemental Figure 2



Supplemental Figure 2: Comparison of the RpoN domain Lys383 in the major groove of the -24 element (left) with the engrailed homeodomain Lys50 (PDB ID: 2HDD) in the major groove of its binding site (right). In each structure, the lysine residue is found in two conformations. Each conformation hydrogen bonds to either a thymine or guanine, which are two basepars apart and on opposite strands of the DNA. Both lysines (blue) and the analogous bases (yellow) are labeled.