Eukaryotic genomes encode an additional layer of genetic information, superimposed on top of the regulatory and coding information, that controls the organization of the genomic DNA into arrays of nucleosomes. We have developed a partial ability to read this nucleosome positioning code and predict the in vivo locations of nucleosomes. Our results suggest that genomes utilize the nucleosome positioning code to facilitate specific chromosome functions including to delineate functional versus nonfunctional binding sites for key gene regulatory proteins, and to define the next higher level of chromosome structure itself.