Layered on top of information conveyed by DNA sequence and chromatin are higher order structures that encompass portions of chromosomes, entire chromosomes, and even whole genomes. Interphase chromosomes are not positioned randomly within the nucleus, but instead adopt preferred conformations. Disparate DNA elements co-localize into functionally defined aggregates or factories for transcription and DNA replication. In budding yeast, Drosophila and many other eukaryotes, chromosomes adopt a Rabl configuration, with arms extending from centromeres adjacent to the spindle pole body to telomeres that abut the nuclear envelope. Nonetheless, the topologies and spatial relationships of chromosomes remain poorly understood. Here we developed a method to globally capture intra- and inter-chromosomal interactions, and applied it to generate a map at kilobase resolution of the haploid genome of Saccharomyces cerevisiae. The map recapitulates known features of genome organization, thereby validating the method, and identifies new features. Extensive regional and higher order folding of individual chromosomes is observed. Chromosome XII exhibits a striking conformation that implicates the nucleolus as a formidable barrier to interaction between DNA sequences at either end. Inter-chromosomal contacts are anchored by centromeres and include interactions among transfer RNA genes, among origins of early DNA replication and among sites where chromosomal breakpoints occur. Finally, we constructed a three-dimensional model of the yeast genome. Our findings provide a glimpse of the interface between the form and function of a eukaryotic genome.