



Figure S1. Genomic distribution of G-quadruplex sequences found using different prediction methods. G4 sequences predicted by three different approaches (G4L1-12: regular expression matching $G_{3-5}N_{1-12}G_{3-5}N_{1-12}G_{3-5}N_{1-12}G_{3-5}$, G4Hunter: sliding window and scoring, and G4-seq: high-throughput *in vitro* detection) were annotated. Genomic features were obtained from the respective annotation files in the 3 species shown. The 3-way overlaps between the different datasets are represented as weighted Venn diagrams (with area-proportional circles or faces for clarity).





Figure S2. Annotation of G-quadruplex sequences found exclusively by G4-seq. The annotations of G4 sequences found exclusively (i.e. no overlaps between the sets) *in vitro* using the G4-seq method (green) were compared to those of the motifs predicted by both the G4Hunter algorithm and G4-seq (purple). Genomic features were obtained from the respective annotation files in the two species shown and are reported on the x-axes. Log₂(enrichment) for each of the assessed features is reported on the y-axes. Permutation tests (n=100 permutations) were performed to assess the significance of the associations; **, *p*-value < 0.01 and |local z-score| > 10; *, *p*-value < 0.05 and |local z-score| > 10.