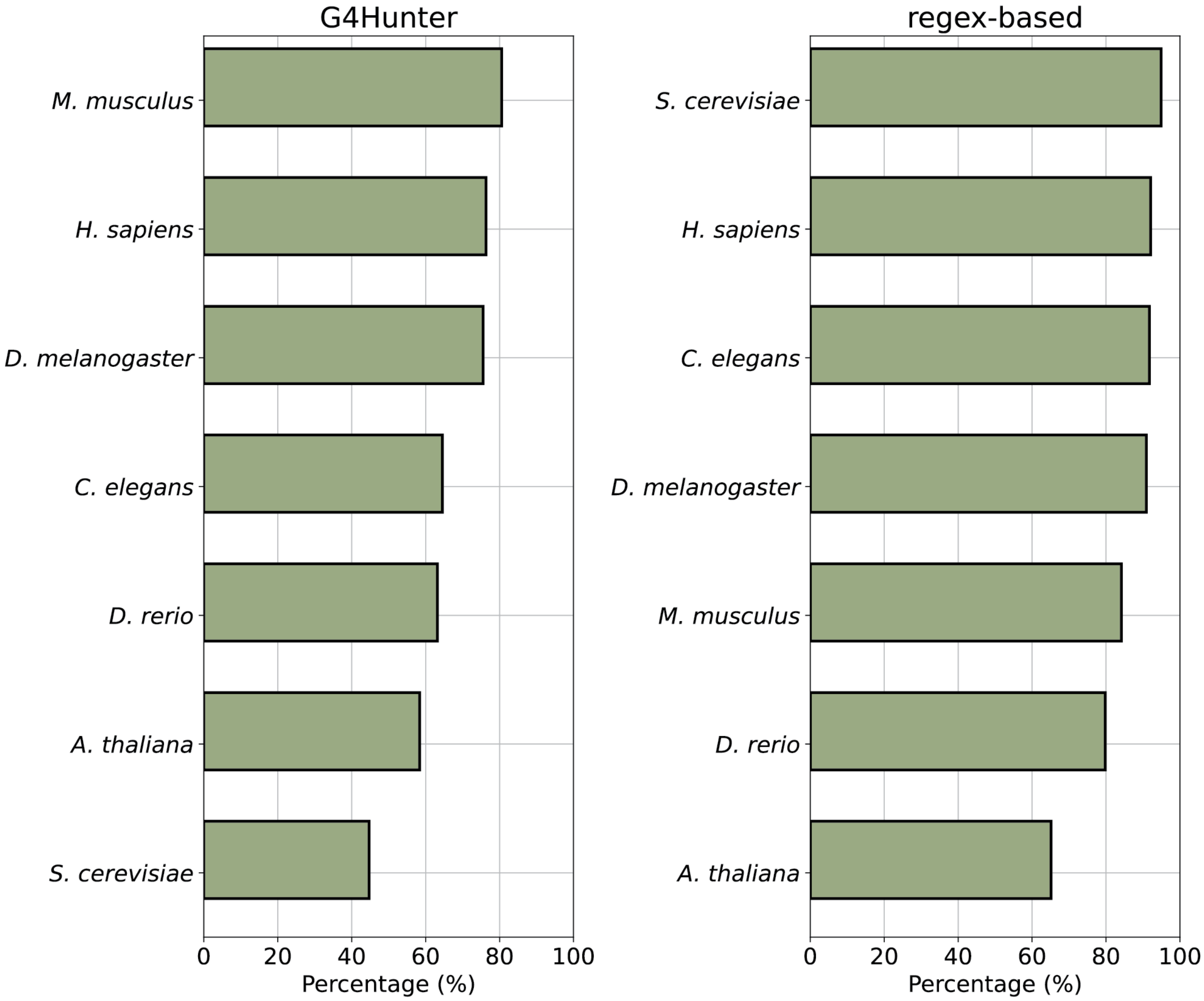
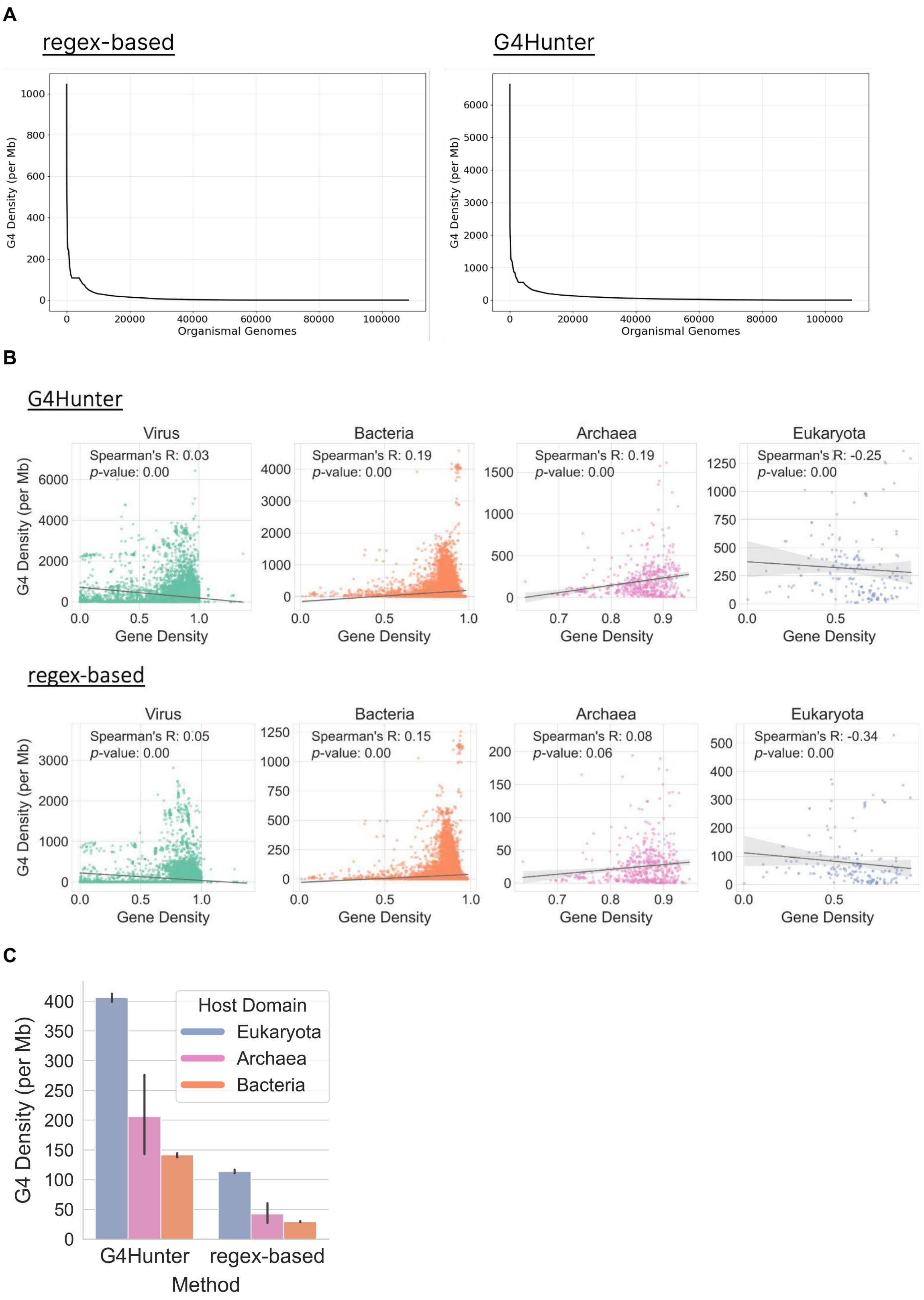
Supplemental Figures

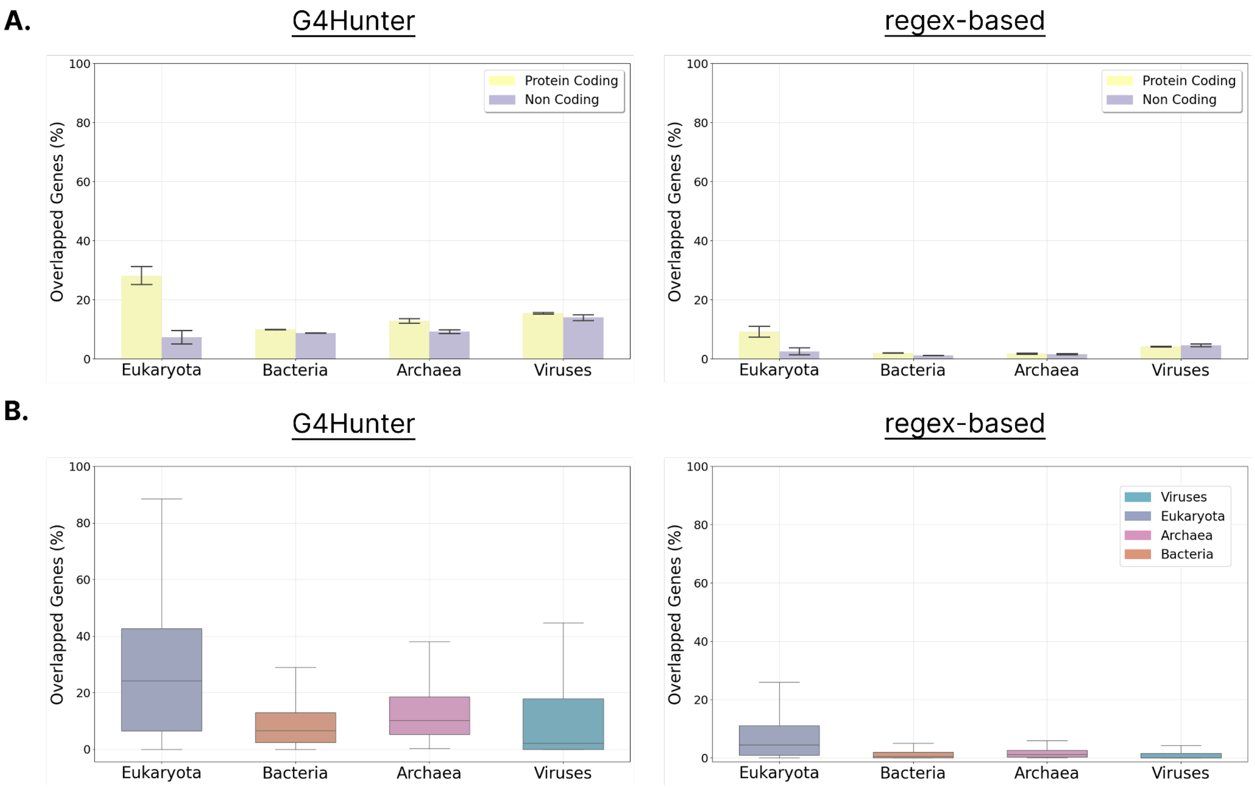


Supplemental Figure 1: Examination of percent overlap between G4s identified with G4Hunter and regex-based approaches and G4-seq data.



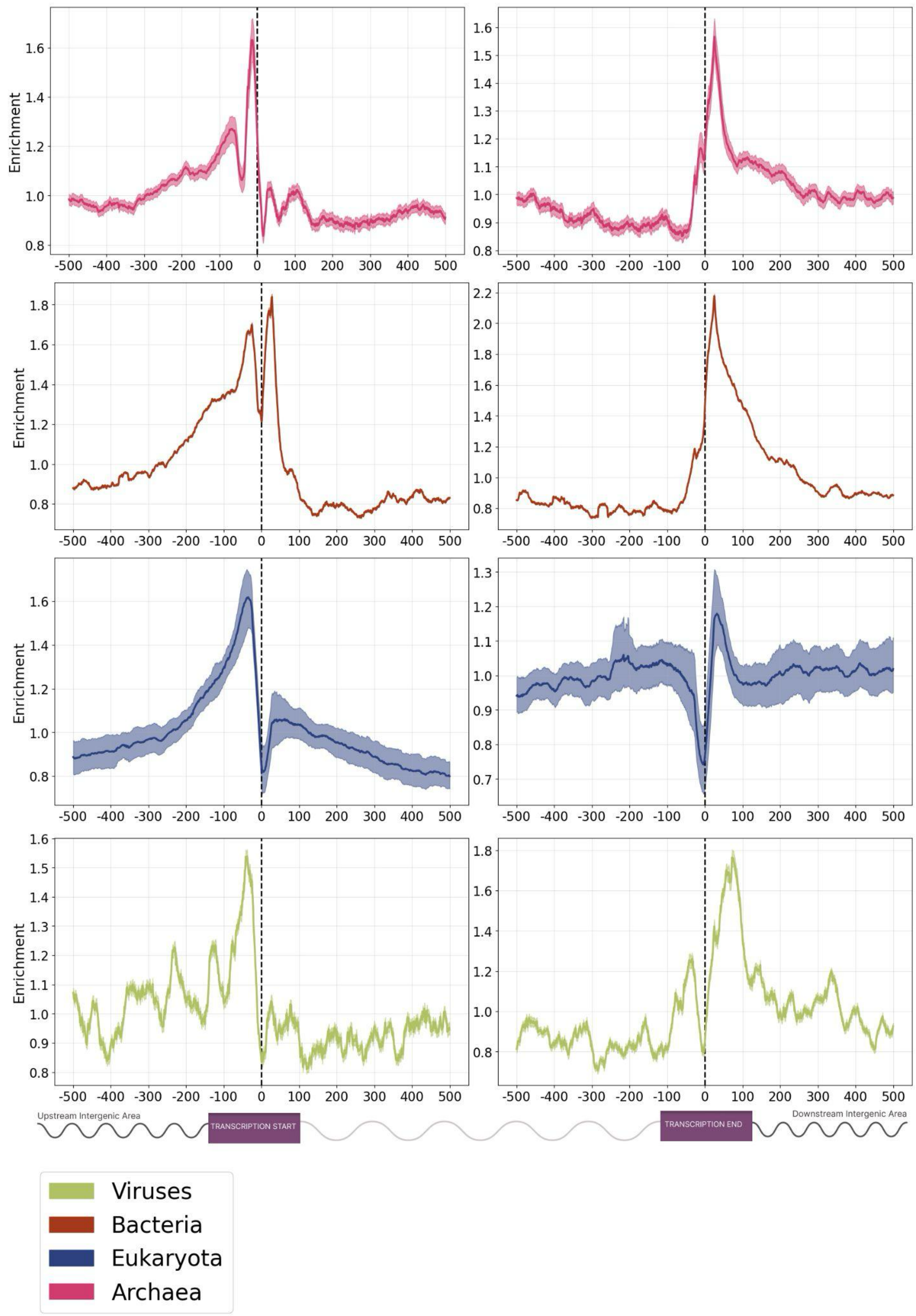
Supplemental Figure 2: Distribution of potential G4 DNA-forming sequences across organismal genomes and taxa. A) G4 motif density per Mb per organismal genome. **B)**

Association between the gene density of a genome and the G4 motif density, stratified by taxonomic subdivision in the three domains of life and viruses. **C)** G4 densities of viral genomes stratified by the domain of their hosts.

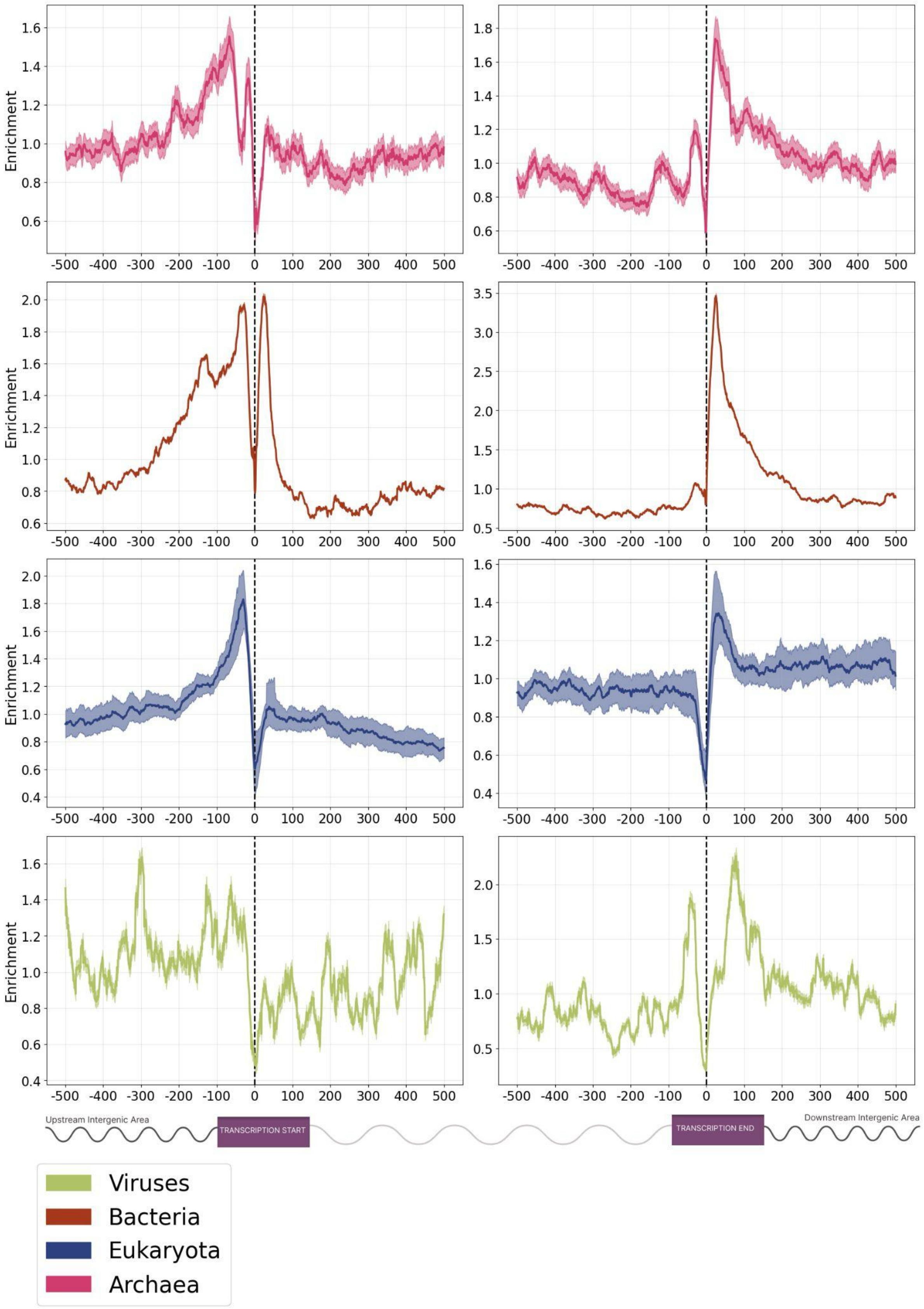


Supplemental Figure 3: Proportion of G4s overlapping genes.

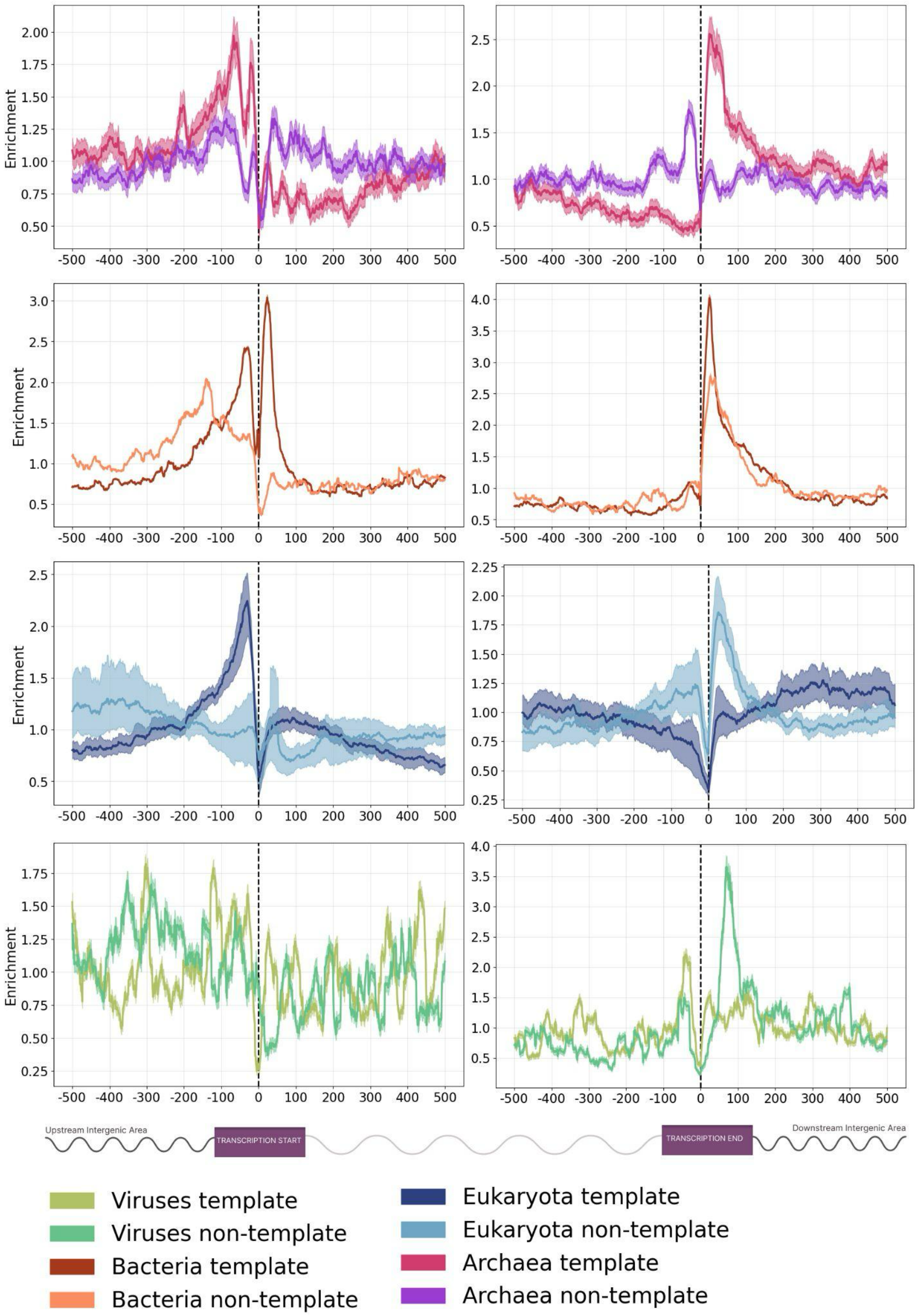
Supplemental Figure 4: Density of G4s in each phylum. Error bars show standard deviation from the mean. All phyla are displayed.


Enrichment
1.6
1.4
1.2
1.0
0.8
1.5
1.3
1.1
0.9
2.2
2.0
1.8
0.7
-500
-400
-300
-200
-100
0
100
200
300
400
500
Upstream Intergenic Area
TRANSCRIPTION START
TRANSCRIPTION END
Downstream Intergenic Area
Viruses
Bacteria
Eukaryota
Archaea

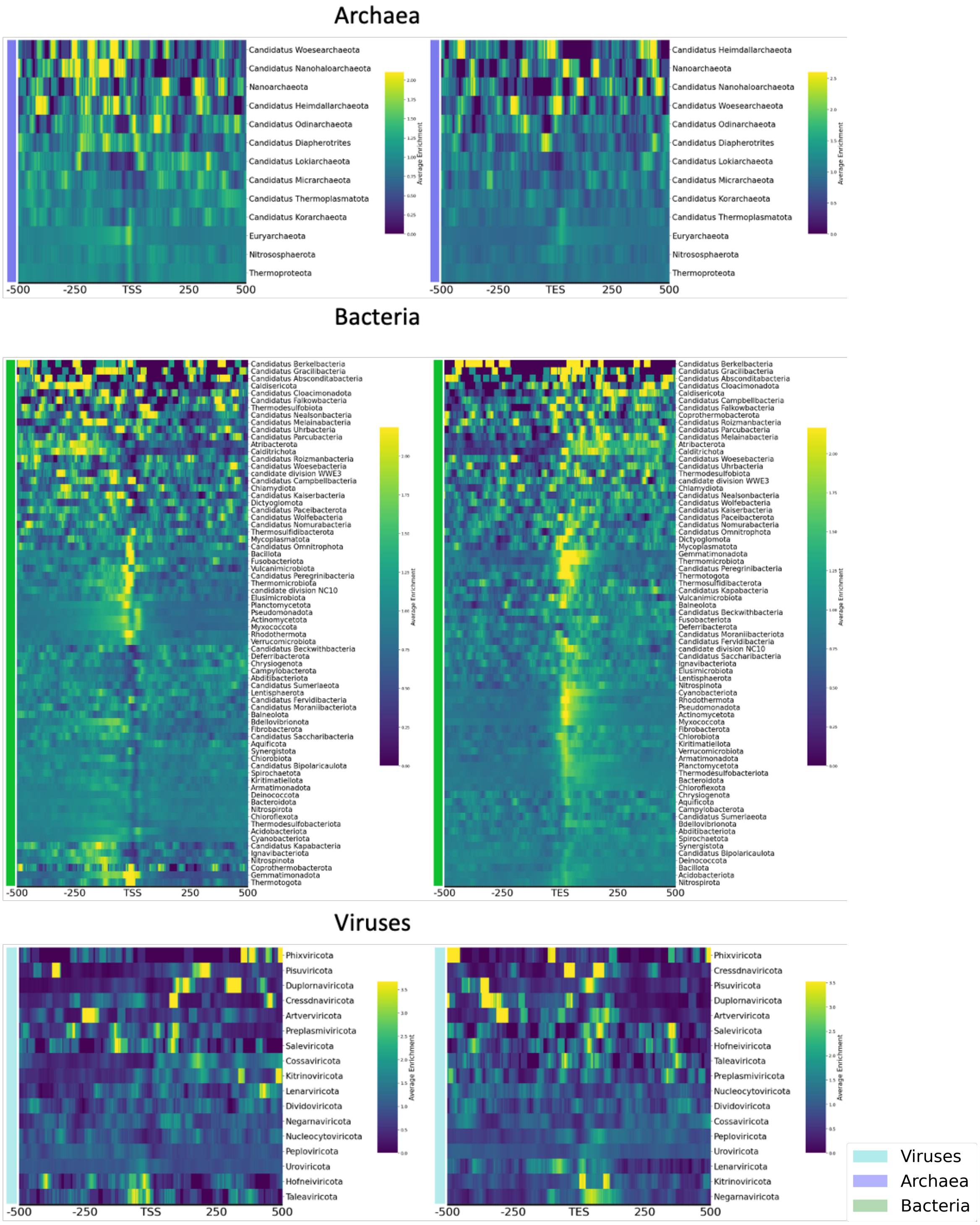
Supplemental Figure 5: The topography of G4s relative to transcription start and transcription end sites across the tree of life. G4 distribution across the three domains of life and viruses derived using the G4Hunter algorithm. Confidence intervals represent the 2.5% lowest and 97.5% highest percentile from Monte-Carlo simulations with replacement (N=1,000).


Enrichment
-500
-400
-300
-200
-100
0
100
200
300
400
500
Upstream Intergenic Area
TRANSCRIPTION START
TRANSCRIPTION END
Downstream Intergenic Area
Viruses
Bacteria
Eukaryota
Archaea

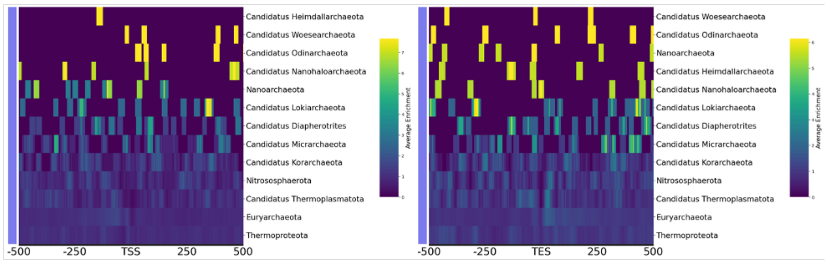
Supplemental Figure 6: The topography of G4s relative to transcription start and transcription end sites across the tree of life. G4 distribution across the three domains of life and viruses derived using the regular expression-based algorithm. Confidence intervals represent the 2.5% lowest and 97.5% highest percentile from Monte-Carlo simulations with replacement (N=1,000).



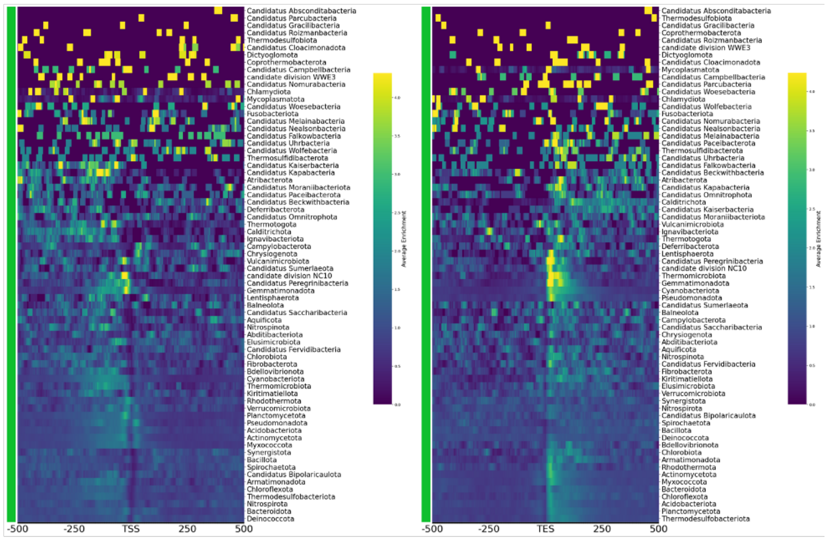
Supplemental Figure 7: The topography of G4s relative to transcription start and transcription end sites across the tree of life at the template and non-template strands. G4 distribution across the three domains of life and viruses derived using the regular expression-based algorithm. Confidence intervals represent the 2.5% lowest and 97.5% highest percentile from Monte-Carlo simulations with replacement (N=1,000).



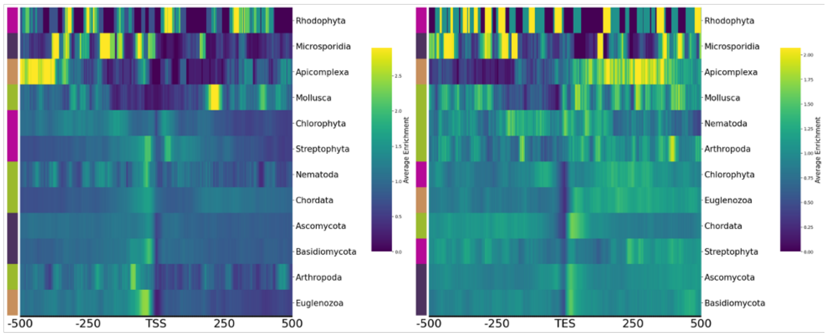
Supplemental Figure 8: Distribution of G4s relative to transcription start and transcription end sites across archaeal, bacterial, and viral phyla. Results shown from the G4Hunter-based algorithm.



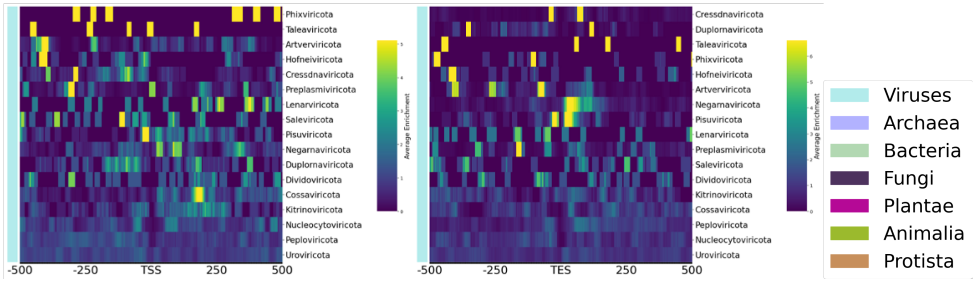
Bacteria



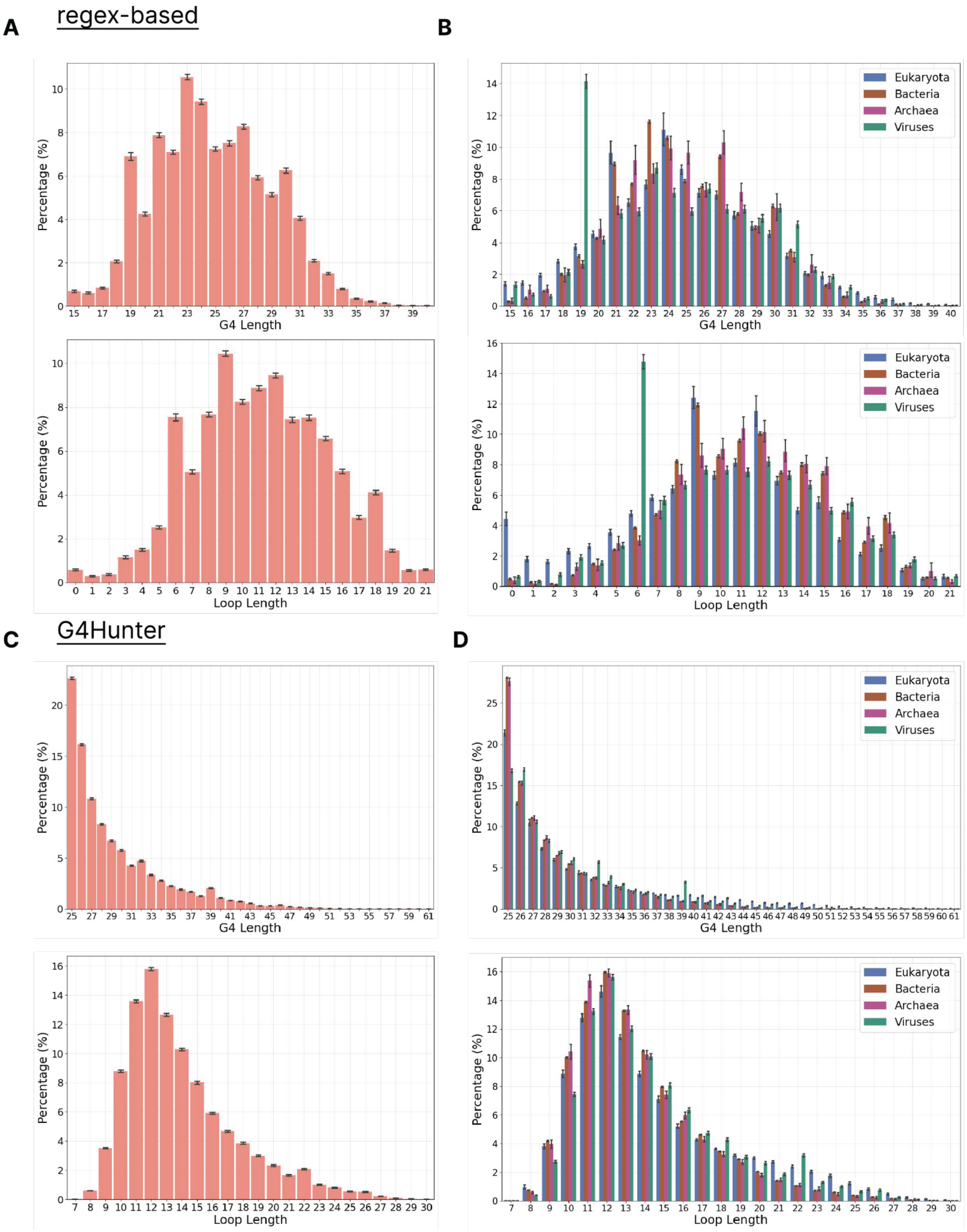
Eukaryotes



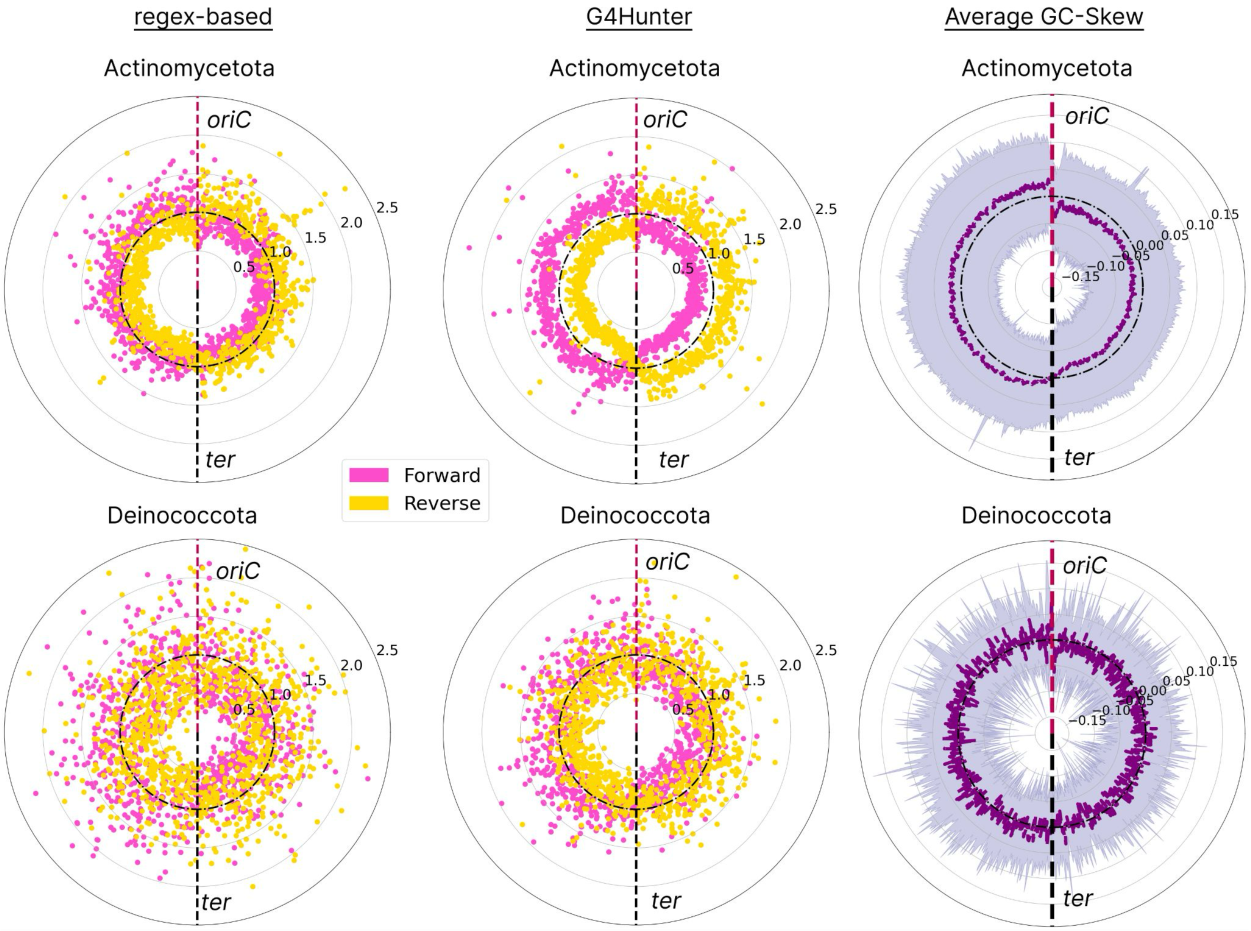
Viruses



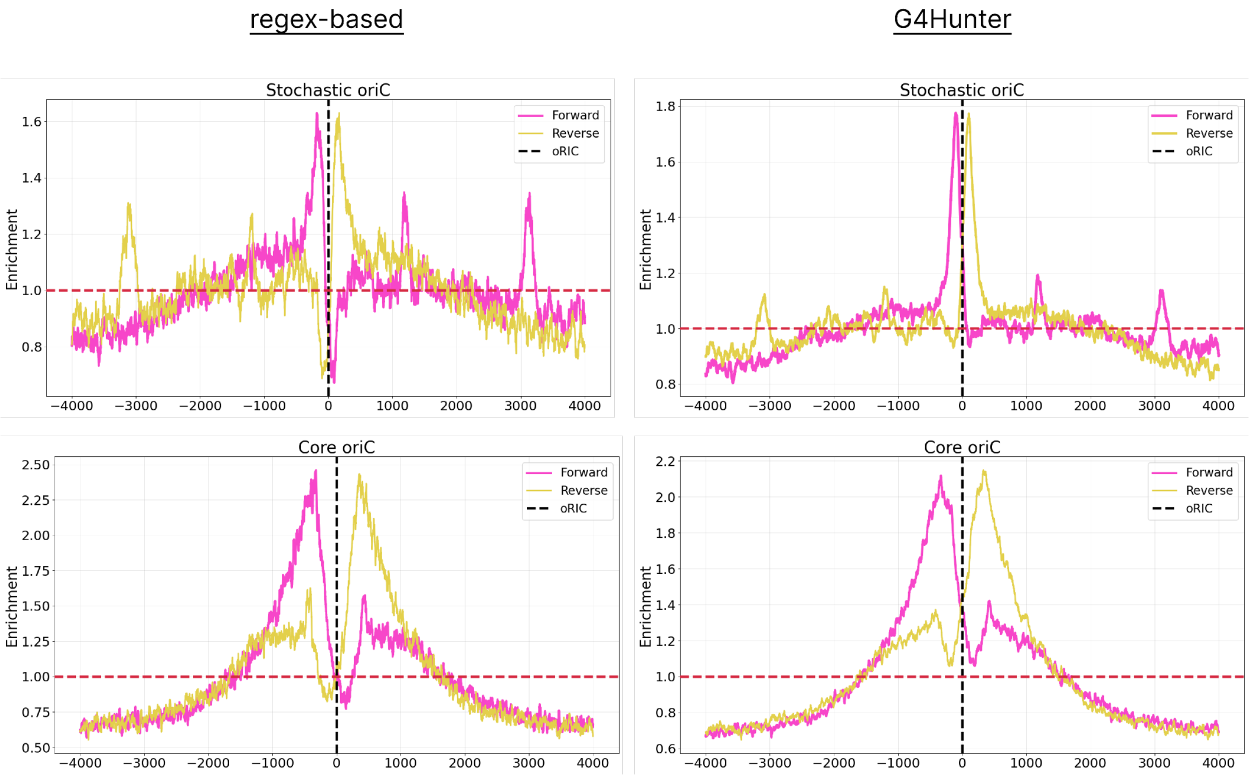
Supplemental Figure 9: Distribution of G4s relative to transcription start and transcription end sites across archaeal, bacterial, and viral phyla. Results shown for G4 motifs derived from the regex algorithm from the regex algorithm.



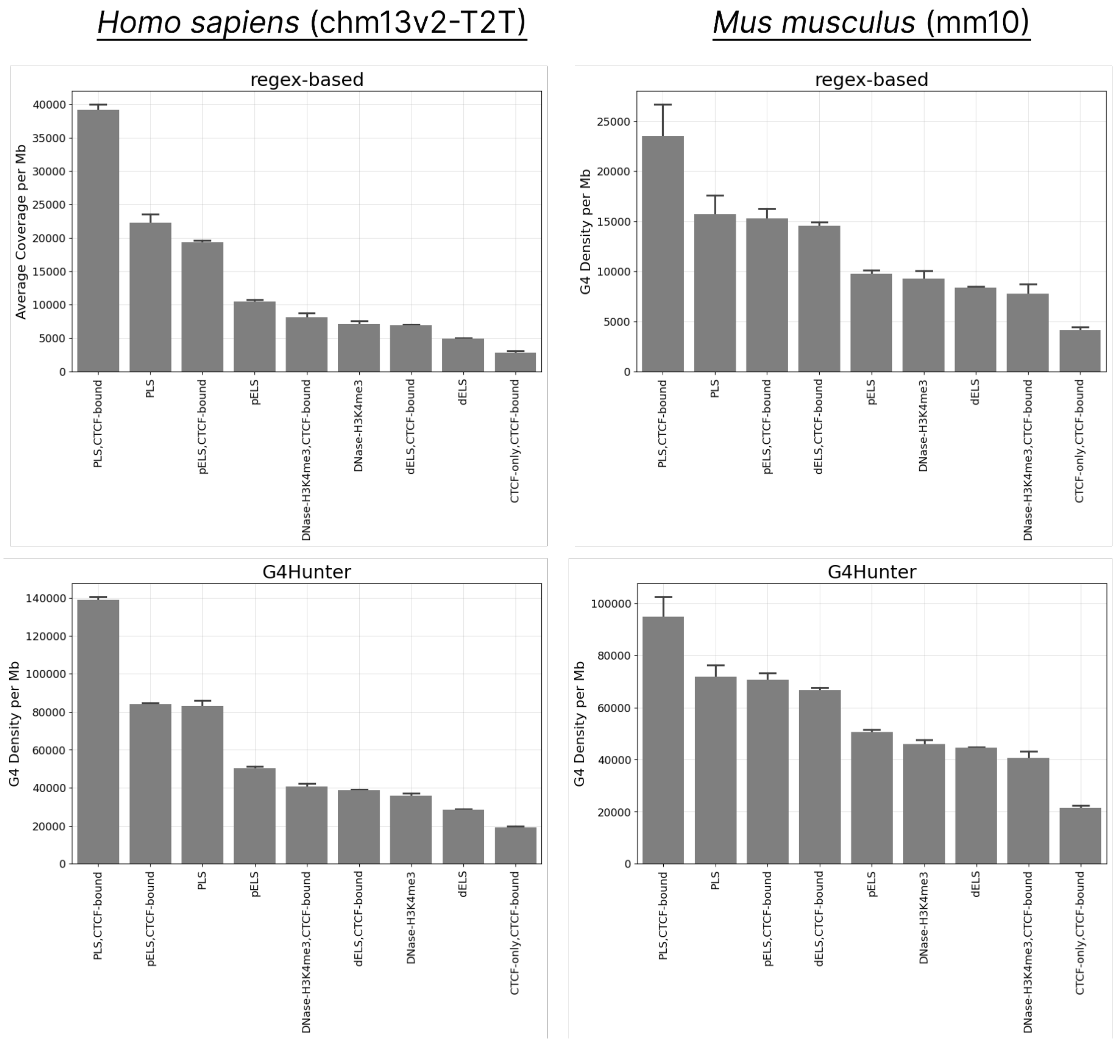
Supplemental Figure 10: Length distribution of G4 lengths and of intervening loops of G4s across taxonomic groups. A-B) Distribution of G-quadruplex sequence length and of intervening loops for the regex algorithm and the G4Hunter algorithm **C-D)** Distribution of G-quadruplex total lengths and loop length for the three domains of life and viruses using the G4Hunter-based algorithm. Error bars represent the standard error.



Supplemental Figure 11: G4 distribution patterns relative to replication origin in bacterial phyla. Results shown for Actionomycetota and Deinococcota. G4s in forward and reverse strand orientation are shown in yellow and pink, respectively. Results shown for G4Hunter-based and regex-based algorithms. Average GC skew is also calculated and shown with purple.



Supplemental Figure 12: G4 distribution relative to origins of replication in *Homo sapiens*. Distribution of G4s relative to the stochastic and core origins of replication for *Homo sapiens* around a window of kb from the origin of replication using both G4Hunter algorithm and the regex-based algorithm.



Supplemental Figure 13: G4 distribution across cis-regulatory elements for *Homo sapiens* and *Mus musculus*. Results shown display the distribution of G4 sequences across various cis-regulatory elements for *Homo sapiens* and *Mus musculus* using both G4Hunter algorithm and the regex algorithm.

Supplemental Table 1: Average G4 motif densities

A. Average G4 motif density by domain (Data for Figure 2B)

Method	**Domain**	**Average G4 Motif Density (per Mb)**
Regex-based algorithm	Archaea	26.41
Regex-based algorithm	Bacteria	29.56
Regex-based algorithm	Eukaryota	82.76
Regex-based algorithm	Virus	65.63
G4Hunter algorithm	Archaea	201.32
G4Hunter algorithm	Bacteria	147.82
G4Hunter algorithm	Eukaryota	327.04
G4Hunter algorithm	Virus	262.54

B. Average G4 motif density in viruses by host domain.

Method	**Host Domain**	**Average G4 Motif Density (per Mb)**
Regex-based algorithm	Eukaryotic	114
Regex-based algorithm	Non-Eukaryotic	29.49
G4Hunter algorithm	Eukaryotic	405.8
G4Hunter algorithm	Non-Eukaryotic	141.65

C. Average G4 motif density in viruses by host domain for results based on G4s from G4Hunter.

Phylum	**Average G4 Motif Density (per Mb)**
Deinococcota	2284.82
Candidatus Bipolaricaulota	1836.32
Chlorophyta	1382.03

Peploviricota	1335.95
Euglenozoa	1112.95
Dividoviricota	971.79
Rhodothermota	759.95
Candidatus Korarchaeota	742.42
Cressdnaviricota	703.71
Myxococcota	665.38
Candidatus Verstraetearchaeota	655.97
Preplasmiviricota	575.64
Gemmatimonadota	553.86
Chordata	532.45
Synergistota	507.41
Basidiomycota	492.67
Candidatus Beckwithbacteria	490.53
Actinomycetota	474.29
Candidatus Binatota	455.43
Verrucomicrobiota	450.42
Armatimonadota	442.03
Chloroflexota	439.41
Microsporidia	437.9
Acidobacteriota	435.66
Thermodesulfobacteriota	425.28
candidate division NC10	424.66
Kiritimatiellota	421.14

Thermomicrobiota	420.15
Cyanobacteriota	406.92
Artverviricota	382.25
Candidatus Latescibacteria	379.82
Cossaviricota	363.86
Kitrinoviricota	363.13
Nitrospirota	351.15
Planctomycetota	349.26
Abditibacteriota	337.96
Chrysiogenota	334.3
Mollusca	330.3
Candidatus Peregrinibacteria	322.59
Candidatus Tectomicrobia	320.2
Candidatus Fervidibacteria	312.13
Candidatus Sumerlaeota	308.6
Annelida	279.17
Nitrospinota	272.9
Saleviricota	260.31
Thermoproteota	260.05
Pisuviricota	247.37
Ascomycota	243.85
Hofneiviricota	233.7
Balneolota	219.6

D. Average G4 motif density in viruses by host domain for results based on the regex-based algorithm.

Phylum	Average G4 Motif Density (per Mb)
Deinococcota	619.76
Peploviricota	566.73
Chlorophyta	518.64
Candidatus Bipolaricaulota	333.71
Euglenozoa	284.06
Cressdnaviricota	235
Dividoviricota	227.6
Chordata	174.13
Myxococcota	164.47
Rhodothermota	144.22
Gemmatimonadota	143.85
Mollusca	133.51
Actinomycetota	131.71
Basidiomycota	113.09
Acidobacteriota	108.65
Candidatus Binatota	107.37
Candidatus Fervidibacteria	94.77
Saleviricota	94.22
Thermomicrobiota	89.11
Chloroflexota	87.12
candidate division NC10	85.37

Preplasmiviricota	84.26
Armatimonadota	82.21
Candidatus Verstraetearchaeota	81.92
Nematomorpha	79.35
Cyanobacteriota	78.49
Thermodesulfobacteriota	75.66
Synergistota	72.37
Annelida	71.6
Planctomycetota	68.82
Candidatus Peregrinibacteria	68.48
Candidatus Korarchaeota	66.64
Verrucomicrobiota	63.08
Cossaviricota	62.31
Kiritimatiellota	61.71
Candidatus Tectomicrobia	60.22
Artverviricota	59.69
Candidatus Latescibacteria	59.15
Microsporidia	54.56
Nitrospirota	53.48
Heterolobosea	50.62
Kitrinoviricota	50.39
Chrysiogenota	49.82
Ascomycota	49.68
Streptophyta	49.49

Candidatus Beckwithbacteria	48.58
Abditibacteriota	42.22
Nitrospinota	40.18
Balneolota	35.4
Arthropoda	35.15

Supplemental Table 2: Phyla and host of the top 100 viral genomes having the highest G4 motif densities.

A.

Phylum	No. of Species	Total no. of G4s	Average G4 Motif Density (per Mb)	Hosts
Peploviricota	73	38836	3489.00	human, vertebrates
Kitrinoviricota	11	384	4905.59	fungi, land plants
Pisuviricota	8	244	3731.96	human, vertebrates
Artverviricota	5	154	3463.75	human, vertebrates
Dividoviricota	1	63	3698.05	
Duplornaviricota	1	20	3801.56	vertebrates, invertebrates
Preplasmiviricota	1	1	3676.47	

B.

Phylum	No. of Species	Total no. of G4s	Average G4 Motif Density (per Mb)	Hosts
Peploviricota	94	38836	3489.00	human, vertebrates
Kitrinoviricota	4	384	4905.59	land plants
Cressdnaviricota	2	244	3731.96	eukaryotic algae, invertebrates, vertebrates

Supplemental Table 3. Selected sequences from the G4 database.

Type	Seq (5′ to 3′)	Length (bp)
regex-based	GGGAAGGGGAGCCGTGGGGTAAAGAAGGG	29
regex-based	GGGGAGTTGGGGGAATAAGGGCGGAGGG	28
regex-based	GGGCTCGGGCTCGGGCTCGGG	21
regex-based	GGGTGCGGGTGCGGGTGCGGG	21
regex-based	GGGATGGGGGCGGGGGCGGG	20
G4Hunter	GGGCTGGGGCGGCTGGTGGTTCTGGG	26
G4Hunter	AGGTTGGTGTGGTGATTGGTGGGGGT	26