The Intricate Relationship of G-Quadruplexes and Bacterial Pathogenicity Islands

Bo Lyu[1,*], Qisheng Song[1,*]

1 Division of Plant Science and Technology, University of Missouri, Columbia, MO 65211, USA

*Corresponding author:

Bo Lyu; E-mail: bl3pt@missouri.edu; Phone number: +1 573 289 3566;

Qisheng Song; E-mail: songq@missouri.edu; Phone number: +1 573 882 9798

Abstract

The dynamic interplay between guanine-quadruplex (G4) structures and pathogenicity islands (PAIs) represents a captivating area of research with implications for understanding the molecular mechanisms underlying pathogenicity. This study conducted a comprehensive analysis of a large-scale dataset from reported 89 pathogenic strains of bacteria to investigate the potential interactions between G4 structures and PAIs. G4 structures exhibited an uneven and non-random distribution within the PAIs and were consistently conserved within the same pathogenic strains. Additionally, this investigation identified positive correlations between the number and frequency of G4 structures and the GC content across different genomic features, including the genome, promoters, genes, tRNA, and rRNA regions, indicating a potential relationship between G4 structures and the GC-associated regions of the genome. The observed differences in GC content between PAIs and the core genome further highlight the unique nature of PAIs and underlying factors, such as DNA topology. High-confidence G4 structures within regulatory regions of *Escherichia coli* were identified, modulating the efficiency or specificity of DNA integration events within PAIs. Collectively, these findings pave the way for future research to unravel the intricate molecular mechanisms and functional implications of G4-PAI interactions, thereby advancing our understanding of bacterial pathogenicity and the role of G4 structures in pathogenic diseases.

Key Words: Guanine quadruplex; Pathogenicity island; Genome; *Escherichia coli*

Introduction

The discovery of the DNA double helix by Watson and Crick in 1953 revolutionized our understanding of genetics and laid the foundation for the modern field of molecular biology (*1*). Nonetheless, the intricate nature of DNA continues to surprise us even today. One such captivating feature is the DNA guanine (G)-quadruplex (G4) structure, a unique arrangement that defies the conventional double helix (*2, 3*). A G4 consists of four guanine bases and is stabilized by Hoogsteen hydrogen bonds. These stacked tetrads are interconnected by loop regions, which can vary in length and sequence, adding further complexity to the structure (Figure 1). It is important to consider the inherent directionality of nucleic acids, with all four strands having the possibility to run in the same 5' to 3' direction, referred to as "parallel", or alternatively, they can run in different directions, known as "antiparallel". G4 regions can be very stable *in vitro*, particularly in the presence of K^+ (*4*). G4 structures are often found in regions of the genome with crucial regulatory functions, such as telomeres, promoters, and enhancers (*2, 5*). These structures play a role in various biological processes, including gene expression, DNA replication, and telomere maintenance (*2, 6*). Further research into G4 structures will undoubtedly uncover new insights into their functions and facilitate the development of innovative technologies.

Pathogenicity islands (PAIs) are genomic regions that contribute to the virulence and pathogenic potential of various microorganisms (*7, 8*). PAIs are distinct segments of the bacterial genome that exhibit unique characteristics compared to the rest of the DNA (*9*). They are often large in size, ranging from tens of kilobases to hundreds of

54 kilobases, and can be integrated into the chromosome or exist as extra-chromosomal
55 elements, such as plasmids. PAIs often exhibit close proximity to tRNA genes,
56 suggesting a putative mechanism where tRNA genes act as anchor points for the
57 integration of foreign DNA acquired through horizontal gene transfer (Figure 1E).
58 One notable feature is their variable GC content, which tends to deviate from the
59 average GC content of the genome in various organisms, such as *Streptomyces* (*10*),
60 *Salmonella* (*11*), and *Yersinia* (*12*). PAIs typically contain clusters of genes involved
61 in pathogenesis, including those encoding secretion systems (e.g., LEE (locus of
62 enterocyte effacement) in *Escherichia coli*), superantigen (e.g., SaPI1 and SaPI2 in
63 *Staphylococcus aureus*), and enterotoxin (e.g., *she* PAI in *Shigella flexneri*). PAIs can
64 be acquired through the transfer of mobile genetic elements, such as plasmids, phages,
65 or integrative and conjugative elements (ICEs), facilitating the incorporation of
66 pathogenicity-associated genes into the recipient genome (*7, 13, 14*). One question
67 raised in PAI is that PAIs often exhibit distinct base composition (G+C contents)
68 compared to the core genome. The underlying reasons for this variation remain
69 unknown, but the preservation of a genus- or species-specific base composition
70 represents a noteworthy characteristic of bacteria (*7*). Herbert Schmidt et al. (2004)
71 proposed a hypothetical mechanism to explain the observed variation, suggesting that
72 factors such as DNA topology and codon message in the virulence regions present
73 could contribute to the preservation of the distinct base composition (*7*). Hopefully,
74 the availability of genome sequences from pathogenic bacteria and their non-
75 pathogenic counterparts presents an exceptional opportunity to explore the intricate

structure variance and underlying mechanisms within PAIs.

Growing evidence has shown that G4 structures exhibit a striking colocalization with functional regions of the genome, and their high conservation across different species suggests a selective pressure to maintain these sequences at specific genomic regions (e.g., genome islands, resistance islands, CpG islands, and PAIs) (*2, 15, 16*). The possibility of interactions between G4 structures and pathogens has been suggested, although this field of study is still in its nascent phase. Some studies observed that bacterial genomes possess G4-forming sequences within their genome regions (*17, 18*). G4 structures are formed by G-rich DNA sequences, and their stability is influenced by the G+C content and arrangement of G tetrads. Interestingly, PAIs often exhibit an altered GC content, putatively contributing to the propensity of G4 structure formation within these regions. The G4 structures in PAIs might modulate the accessibility of transcription factors, DNA-binding proteins, or RNA polymerase in pathogens, as documented in eukaryotes (*2, 19*), thereby influencing the expression of virulence-associated genes (*20*). The formation of G4 structures within PAIs may serve as an additional layer of regulation that fine-tunes the expression of genes critical for pathogenesis. Hence, the investigation of G4 structures within PAIs may open new avenues for the development of therapeutic strategies aimed at disrupting the regulatory mechanisms of pathogenicity-associated genes.

Results

98 **Genomic information, PAI patterns, and the presence of G4 structures in 89**
99 **reported pathogenic strains**

100 A dataset of PAIs was compiled from 89 reported pathogenic strains of bacteria,
101 encompassing 222 distinct types of PAIs. Pathogens exhibiting similar PAIs displayed
102 closely clustered patterns on phylogenetic branches, such as LEE in *E. coli* strains
103 (Figure 2A). Additional information, including the genome length (bp), G+C content
104 (%), rRNA density, tRNA density, and PAI length (bp), was present and showed
105 conserved patterns in the same species (Figure 2A & Supplementary file 1a). PAIs
106 commonly exhibit mosaic-like patterns, exemplified by the presence of distinct PAIs
107 like FPI in *Francisella tularensis*, SaPIbov in *Staphylococcus aureus*, and Hrp PAI in
108 *Xanthomonas campestris* (Figure 2B). Many PAIs were present associated with
109 tRNAs, such as the insertions of $tRNA^{Thr}$, $tRNA^{Phe}$, and $tRNA^{Gly}$ in *E. coli* strains
110 (Figure 2B & Supplementary file 1b). The presence of PAIs distributes in similar
111 genomic regions across different pathogens or strains, showing non-random patterns
112 and functionally clustered. Employing the G4Hunter search algorithm, the study
113 identified a total of 225,376 putative G4 sequences in these 89 pathogenic genomes
114 (Supplementary file 1a). The heatmap also showed that the number of G4 structures
115 was diverse in the pathogen genomes (Figure 2C).

116 **Interaction between PAIs and G4 structures in different genomic features**

117 The analysis of G4 structures across all pathogen species demonstrated a positive
118 correlation between the number of G4 structures and the GC content in various
119 genomic features, including the whole genome, gene, promoter, rRNA, and tRNA

120 regions (Figure 2D). The frequency of G4 structures, measured as the frequency of
121 predicted G4-forming sequences per 1000 base pairs (bp), also showed a positive
122 correlation with the GC content across the analyzed genomic elements (Figure 2E). A
123 G4 score of 1.4 and 1.6 consistently supported a positive correlation between the
124 number and frequency of G4 structures and the GC content across diverse genomic
125 features (Figure 2-figure supplement 1). Additionally, this study observed that the GC
126 contents in the genome region were significantly higher compared to the
127 corresponding PAIs region that was classified into five parts according to the genome
128 datasets (Figure 3A-E). Nonetheless, this study noted a unique pattern in the
129 frequency of G4 structures within diverse regions of the PAIs, particularly in regions
130 with GC contents less than 30% and greater than 60%.

131 **Putative functions of G4 structures in PAIs**

132 The study used *E. coli* as an example to investigate the potential regulatory role and
133 function of genes covered by G4 structures in PAIs. *E. coli* contains at least ten types
134 of PAIs in different strains, and one of the well-known PAIs is LEE (Figure 3F),
135 harboring genes responsible for causing attaching and effacing lesions (*21, 22*). One
136 stable G4 structure with a G4Hunter score of 1.6 was identified at position 37,085 in
137 the LEE PAI of *E. coli* str. O103:H2 12009 (Figure 3G), located between an IS
138 element and a tRNA insertion site. The tRNA region generally contains a higher G4
139 frequency compared with transfer-messenger RNA (tmRNA) and rRNA regions in the
140 bacterial genome (*23*). Interestingly, this G4 structure found in *E. coli* str. O103:H2
141 12009 was present in close proximity to a tRNA region, suggesting a potential

142 regulatory role of G4 structures in the tRNA gene, or upstream- and downstream-
143 genes that are responsible for LEE virulence. Additionally, another stable G4
144 sequence with a score of 1.381 was discovered at position 12,457 in the *E. coli* str.
145 CFT073 PAI II to provide more evidence of G4 in PAI regions (Figure 3H).
146 Functional enrichment analysis was conducted to explore the putative functions of
147 G4-covered genes in the two *E. coli* strains (Supplementary file 1c & 1d). The results
148 revealed that the genes covered by G4 structures were predominantly involved in
149 genetic information processes, including DNA binding, DNA integration, and nucleic
150 acid metabolism processes (Figure 3I & J).
151

152 **Discussion**

153 This study found that the non-random distribution of G4 structures within PAIs across
154 different bacterial species, signifies a potential regulatory role in bacterial
155 pathogenicity. The conservation of G4 structures within the same pathogenic strains
156 suggests a crucial and possibly conserved function in regulating pathogenic traits. The
157 findings are similar to previous reports that showed that the G4 structures display
158 uneven distribution patterns in eukaryotic and prokaryotic genomes and are conserved
159 evolutionary groups (*23-25*). To understand the origin of G4 structures within PAIs,
160 we hypothesized that these G4 sequences could be acquired through three types of
161 horizontal gene transfer mechanisms: conjugation, transformation, and transduction
162 (Figure 3K). These mechanisms serve as means for genetic material exchange
163 between different organisms. Considering the presence of G4 sequences within the

164 PAIs, it is plausible that these sequences are transferred along with the PAIs through
165 these horizontal gene transfer mechanisms. Additionally, the presence of G4
166 structures within the promoter, rRNA, and tRNA regions may have functional
167 implications for the regulation of DNA replication, ribosome biogenesis, protein
168 synthesis, and other RNA-related processes (*6, 26, 27*). Throughout evolution, there
169 seems to be a greater frequency of G4 structures in regulatory genes, such as the
170 tRNA region, compared to other genes, enabling intricate control of gene expression
171 in signal transduction pathways (*28*).

172 The study found that the genomic regions surrounding the PAIs (i.e., core
173 genome) tend to have a higher GC content than PAI regions, which was consistent
174 with the fact that PAIs often exhibit distinct base compositions compared with the
175 core genome (*7*). The variation was explained by the presence of G4 sequences within
176 the PAIs, whereas the results were surprising. This study observed a distinct pattern in
177 the frequency of G4 structures within different regions of the PAIs. This differential
178 distribution of G4 structures suggests that (i) specific genomic segments within the
179 PAIs may be more prone to induce G4 formation discrepancy; (ii) the variation of
180 base composition between core genome and PAIs is partially correlated with the
181 presence of G4 structures; (iii) the frequency of G4 structures in PAIs present stable
182 as the core genome in the most situation; (iv) an alternative hypothesis, other factors,
183 such as i-motif (i.e., the anti-G4 structure) and CpG island, may work synergistically
184 with G4 and potentially contribute the base composition variation (*29, 30*).

185 Enrichment analysis indicated a predominant involvement of these G4-covered
186 genes in genetic information processes, encompassing DNA binding, DNA
187 integration, and nucleic acid metabolism. This suggests that G4 structures may play a
188 regulatory role in these essential cellular processes, especially gene expression and
189 DNA-related functions. For instance, G4 structures in the promoter regions of certain
190 transcription factors may influence their binding affinity to DNA and subsequently
191 affect downstream gene expression patterns (*31, 32*). These elements frequently
192 utilize DNA integration mechanisms mediated by integrases, recombinases, or
193 transposases to transfer or incorporate genetic material into the bacterial genome (*33,*
194 *34*). One compelling illustration is a study that identified a 16-base pair cis-acting G4
195 sequence near the pilin locus in *Neisseria gonorrhoeae*, demonstrating its pivotal role
196 in antigenic variation and directing recombination to a specific chromosomal locus
197 (*20, 35*). Disruption of the G4 structure in this context impeded pilin antigenic
198 variation and recombination, highlighting its significance in immune evasion
199 mechanisms. Additionally, considering the distance between G4 structures and the
200 beginning site of gene (e.g., transcription start site (TSS)) in the analysis of promoter
201 regions is pivotal for a comprehensive understanding of their regulatory impact on
202 gene expression (*5*). The spatial proximity to the TSS influences interactions with
203 regulatory elements, potentially modulating the binding of transcription factors and
204 RNA polymerase. This spatial relationship affects accessibility, with G4 structures
205 closer to the TSS potentially acting as direct impediments to transcription initiation.

206 Acknowledging these spatial nuances would provide crucial insights into the
207 functional implications of G4 structures in promoters.

208 Overall, the conserved evolutionary relatedness of PAIs, the detection of stable
209 G4 structures in specific genomic positions, and the enrichment of G4-covered genes
210 in genetic information processes collectively support the hypothesis that G4 structures
211 may have regulatory functions in key biological processes in pathogens. However, it
212 is important to acknowledge and address certain limitations that could potentially
213 affect the interpretation of the results. One such limitation is the reliance on genome
214 sequences obtained from external laboratories and datasets, which introduces a level
215 of uncertainty regarding the accuracy and completeness. Furthermore, the dynamic
216 nature of bacterial genomes, including genetic rearrangements and horizontal gene
217 transfer events, can complicate the accurate assembly and annotation of genome
218 sequences. Lastly, the stability of G4 structures seems to be important for their
219 function according to recent evidence (*36*). Hence, exploring the relationship between
220 G4 stability and function is a valuable and intriguing topic that could provide insights
221 into the nuanced ways G4 structures contribute to cellular processes and potentially
222 offer new avenues for therapeutic interventions or molecular engineering. To
223 overcome these constraints, fostering collaboration among research teams and
224 participating in data-sharing endeavors becomes imperative to guarantee access to
225 high-quality genome data for exhaustive analyses. Moreover, it is crucial to interpret
226 the results with caution and continue refining this understanding through validation
227 experiments and collaborative efforts.

228

229 # Methods

230 **Selection and extraction of DNA Sequences**

231 A total of 89 genomes corresponding to the identified pathogens from the
232 Pathogenicity Island Database (PAIDB) were included in the study. The complete
233 bacterial genomic DNA sequences and their corresponding annotation files in .gff
234 and .fna formats were obtained from the Genome database of the National Center for
235 Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/genome). To
236 ensure the reliability and completeness of the dataset, only completely assembled
237 genomes were included in the analysis. To avoid redundancy and incomplete
238 sequences, one representative genome was selected for each species or strain. The
239 selection of representative genomes was based on a careful examination of the
240 supplementary material (Supplementary file 1a) accompanying the study. TBtools
241 (https://cj-chen.github.io/tbtools), a versatile bioinformatics tool with extensive
242 applications in both eukaryotes and prokaryotes (*37, 38*), was employed for extracting
243 genomic sequences. This tool facilitated the retrieval of gene regions, promoters (2 kb
244 upstream of the genes), tRNA regions, and rRNA regions from the selected genomes.
245 PAI regions were downloaded following previously documented information in
246 PAIDB (Supplementary file 1a & 1b). Default thresholds and parameters were applied
247 during extraction to maintain consistency across all genomes.

248 **Data process and detection of G4 structures in genomic features**

249 The G4Hunter algorithm, a widely used tool for G4 prediction, was employed for the
250 identification of G4 motifs in the genomic sequences (*39*). The G4Hunter parameters
251 were set to a window size of "25" and a G4 score threshold of 1.2, which ensured the
252 identification of potential G4 sequences (*23, 40*). The study additionally utilized G4
253 scores of 1.4 and 1.6 as a means of cross-verification for the results. The study
254 quantified the predicted number of putative G4-forming sequences within different
255 genomic features, including the whole genome, gene, promoter, tRNA, rRNA, and
256 PAI regions. The density of G4 motifs was determined by dividing the number of G4
257 sequences by the total length of the genome, while the length ratio of G4 motifs was
258 calculated by dividing the total length of the G4 sequences by the total length of the
259 genome.

260 **Relationship between G4 structures and PAIs**

261 The heatmap was used to show the distribution of G4 motifs in the genome divided by
262 ten parts as PAI regions using R package “pheatmap”. The correlation between the
263 number of G4 structures and the GC content was analyzed across various genomic
264 elements, including the whole genome, gene, promoter, rRNA, and tRNA regions.
265 The analysis utilized the *R*-squared value (R^2) to determine the fit goodness of the
266 correlation. The correlation's significance was evaluated through *p*-values along with
267 a 95% confidence interval. Subsequently, a ROC analysis, yielding an area greater
268 than 0.90, was employed to quantify sensitivity and specificity. The GC content in the
269 genome regions and corresponding PAI regions was compared and classified into
270 different ranges to explore the variation in base composition. GraphPad Prism (V.5.02,

271 GraphPad Software, Inc) was employed to conduct Normality and Lognormality Tests.
272 The K-S test and F-test were used to assess normal distribution and variances, and
273 Student's t-test was used to identify significant differences.

274 **Phylogenetic Tree Construction**

275 The exact Taxonomy ID (taxid) for each analyzed group was obtained from the NCBI
276 Taxonomy Database using the Taxonomy Browser. The Neighbor-Joining (NJ)
277 method was employed to construct the phylogenetic trees for the analyzed groups.
278 The phylogenetic trees were generated using MEGA11 software
279 (www.megasoftware.net), which offers robust algorithms and comprehensive tools for
280 phylogenetic analysis. To assess the reliability and statistical support of the
281 phylogenetic tree branches, bootstrap analysis was performed. One thousand
282 bootstrap replicates were used to estimate the confidence levels of the branching
283 patterns in the phylogenetic trees. The phylogenetic trees, along with the bootstrap
284 support values, were displayed and visualized using the Interactive Tree of Life
285 (ITOL) platform (https://itol.embl.de/).

286 **Gene functional annotation**

287 The gene sequences covered by G4 structures within PAIs were subjected to gene
288 ontology (GO) annotation (https://geneontology.org/). The gene sequences were
289 translated into protein sequences using the Expasy online toolkit
290 (https://web.expasy.org/translate/). This tool performs the translation based on the
291 standard genetic code, converting the DNA nucleotide sequence into its
292 corresponding amino acid sequence. The GO annotation database assigned GO terms

293 to the protein sequences based on their predicted functions and known biological
294 process (BP), molecular function (MF), and cellular component (CC). Fisher's exact
295 test was employed to determine the statistical significance of the enrichment results.
296 The obtained *p*-values indicated the overrepresentation of specific GO terms, with
297 lower p-values suggesting higher significance.

298 **Statistics and reproducibility**

299 All genomic data utilized in this study, including the species-specific datasets, were
300 obtained from publicly available sources. Statistical analyses, such as the Kruskal-
301 Wallis test, F test, Student's t-test, Wilcoxon test, and chi-square test, were performed
302 using GraphPad Prism software. The samples used in the statistical analyses
303 corresponded to the genomic data, PAIs, or specific genes under investigation.

304

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Competing Interests

The authors declare no competing interests.

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Credit author statement

Bo Lyu: writing original draft, data analysis, and visualization.

Qisheng Song: writing original draft-reviewing and supervision.

Ethical approval

Ethical review and approval were not required for this study.

Availability of data and materials

The original reported PAIs datasets analyzed in this study are available from the publication with the DOI: 10.1093/nar/gku985. Additionally, supplementary materials accompanying this article provide further PAIs data analyzed in the study. These supplementary materials contain detailed information and datasets that support the findings and conclusions presented in this research. Researchers and interested individuals can access supplementary materials to explore and validate the results obtained in this study.

Consent for publication

Not applicable.

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441

Figure caption

Figure 1. Structural and Functional Aspects of G-quadruplex (G4) Structures and Pathogenicity Islands (PAIs). (A) Schematic representation of a guanine tetrad stabilized by Hoogsten base pairing and a positively charged central ion, illustrating the key elements of G4 structures. (B) Structural heterogeneity of G4 structures. G4 structures exhibit polymorphism and can be categorized into different families, such as parallel or antiparallel, based on the orientation of the DNA strands. They can fold either intramolecularly or intermolecularly, leading to diverse structural configurations. (C) General sequence formula for G4, highlighting the repeated occurrence of guanine-rich sequences that form G4 structures. (D) Regulatory roles of G4 in transcription. G4 can regulate transcription by blocking RNA polymerase from binding to promoter sequences or aiding in single-stranded DNA (ssDNA) formation, thereby enhancing transcription. (E) General structure of PAI. PAIs are characteristic regions of DNA found within the genomes of pathogenic bacteria, distinguishing them from nonpathogenic strains of the same or related species. Repeat sequences are DNA segments duplicated within the PAI and can serve as recognition sites for various enzymes involved in the integration and excision of the PAI from the bacterial chromosome. tRNA genes act as anchor points for the insertion of foreign DNA acquired through horizontal gene transfer. Virulence genes encode proteins or factors that play crucial roles in the virulence and pathogenicity of the bacterium, contributing to adhesion, invasion, immune evasion, toxin production, or other pathogenic mechanisms. Insertion elements include transposons, bacteriophages, or

23 plasmids, enabling the PAI to be transferred between bacterial cells and potentially
24 disseminated to different strains or species.

25

26 **Figure 2. Analysis of Pathogenicity Islands (PAIs) and G-quadruplexes (G4) in**
27 **Pathogen Genomes.** (A) Phylogenetic analysis of pathogen genomes based on 89
28 bacterial strains, showing the evolutionary relationships among species. Additional
29 genomic information, including genome size, GC content, rRNA density, tRNA
30 density, and PAI length, is provided. The same color indicates the same species. (B)
31 Genomic location of specific PAIs in bacterial genomes, divided into ten regions.
32 PAIs are represented by green triangles, and their names are indicated. The tRNA
33 insertion sites are also marked. (C) Heatmap illustrating the relative abundance of G4
34 structures in bacterial genomes, divided into ten regions. Red indicates a higher
35 relative abundance, while blue indicates a lower relative abundance. (D & E)
36 Correlation analysis between the number of G4 structures, the frequency of G4
37 structures and GC content in various genomic features, including the whole genome,
38 genes, promoters, rRNA, and tRNA.

39

40 **Figure 2-figure supplement 1.** Correlation analysis between the number of G4s, the
41 frequency of G4s and GC content in various genomic features, including the whole
42 genome, genes, promoters, rRNA, and tRNA, using G4 scores of 1.4 and 1.6.

43

44 **Figure 3. Comparison and Functional Annotation of G-quadruplexes (G4) within**
45 **Pathogenicity Islands (PAIs).** (A-E) Comparison of GC content (left panel) and GC
46 frequency (right panel) between the genome and PAIs, categorized into five regions
47 (20%-30%, 30%-40%, 40%-50%, 50%-60%, and 60%-70%). */**/***/**** indicates
48 significant difference ($p < 0.05/0.01/0.001/0.0001$). (F) Evolutionary relatedness of 10
49 types of PAIs (categorized into six main categories) in *E. coli* strains. (G & H)
50 Examples of G4 structures within PAIs in *E. coli* strains. The grey bar represents the
51 virulence region, the red box indicates a virulence gene, the blue box represents an
52 insertion site region or repeat, the green box denotes an integrase, the purple triangle
53 indicates a tRNA insertion site, and the yellow triangle indicates an effector. (I &J)
54 Functional annotation analysis of G4-covered genes within PAIs in two *E. coli* strains,
55 including biological process (BP), cellular component (CC), and molecular function
56 (MF) categories. (K) Hypotheses on the origin of G4 structures within PAIs,
57 involving gene horizontal transfer mechanisms (conjugation, transduction, and
58 transformation).

59

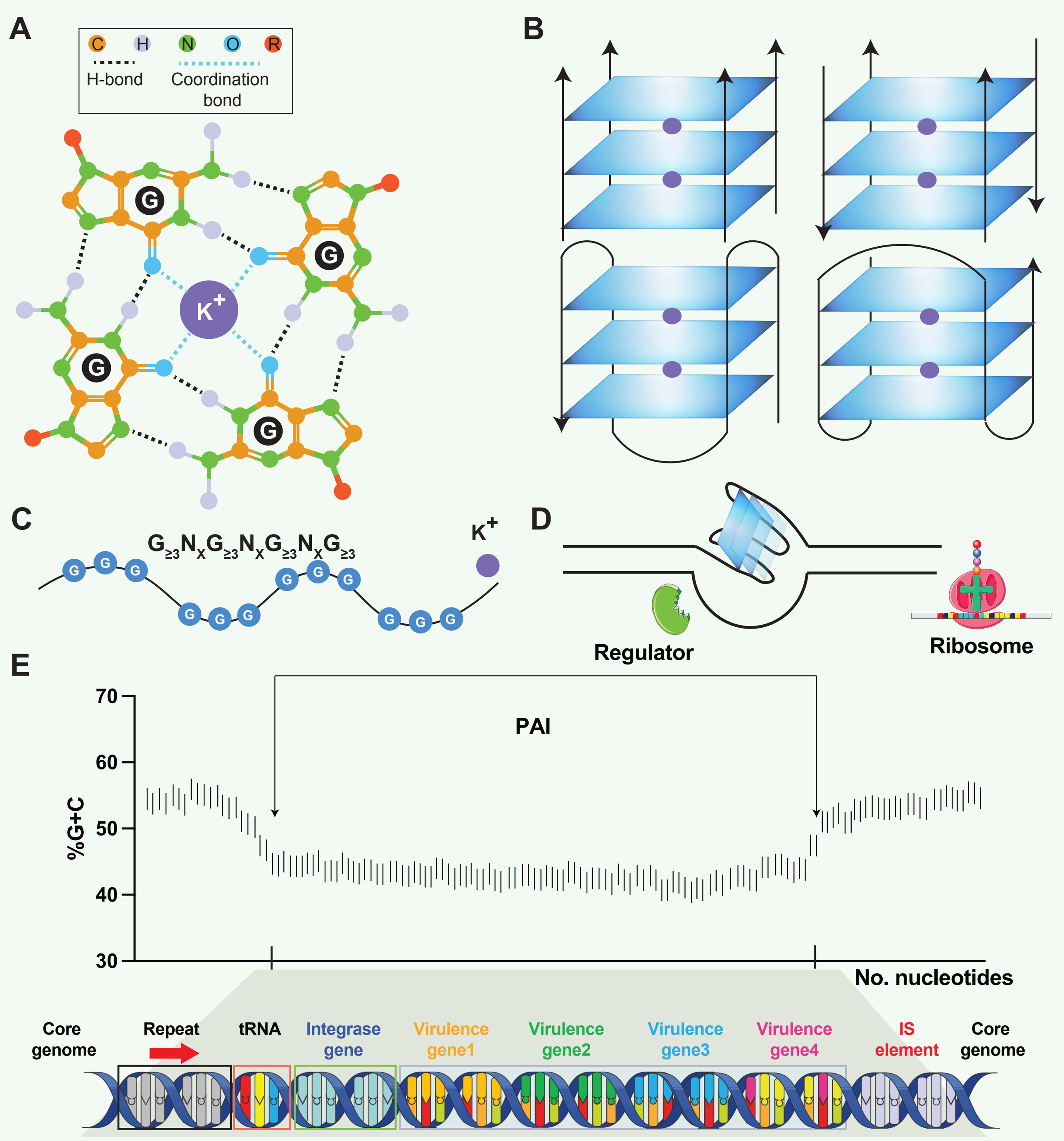
60 **Supplementary File 1**

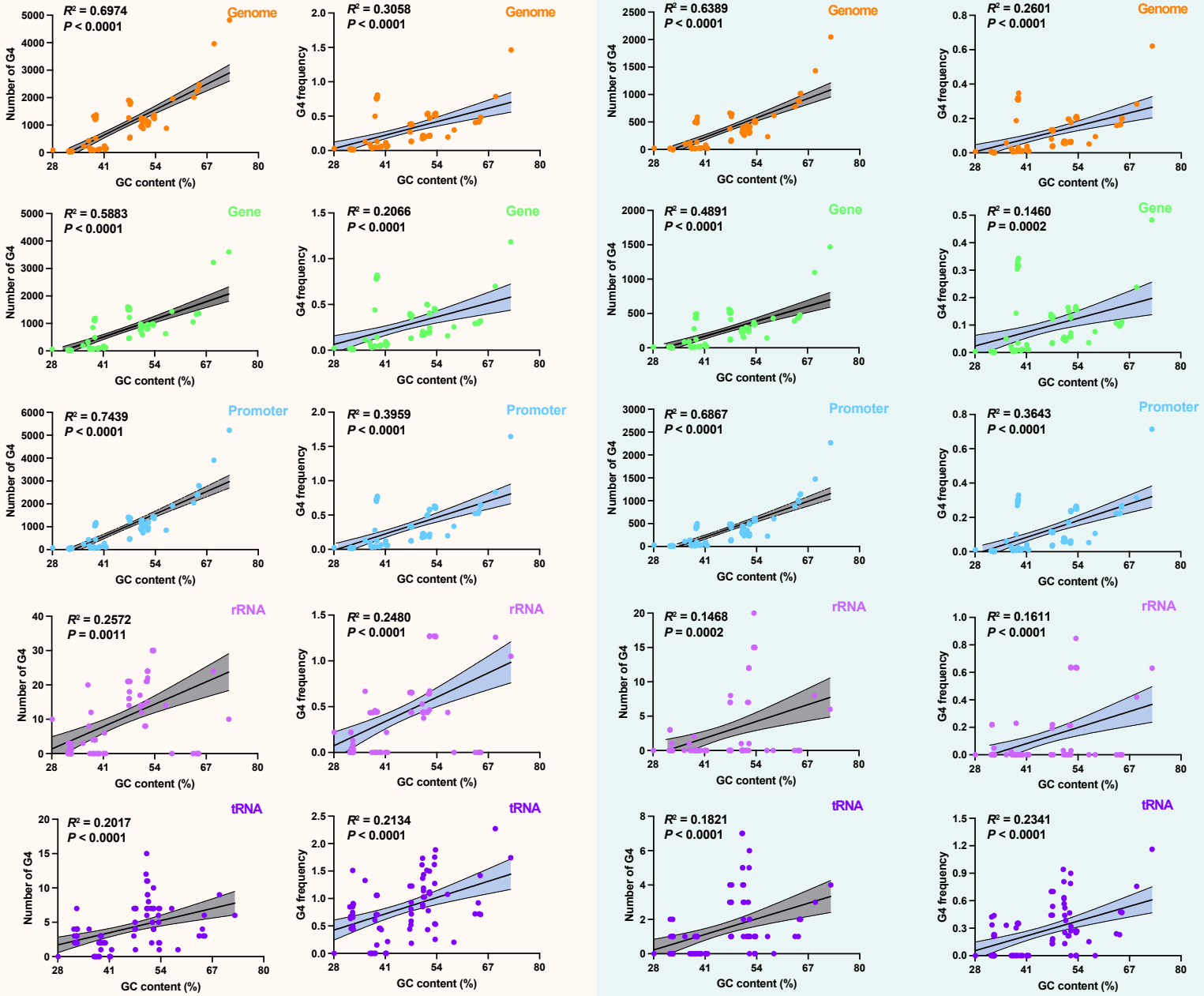
61 **Supplementary File 1a.** List of 89 bacterial species or strains within the same species
62 and the number of G4s within their genomic features.

63 **Supplementary File 1b.** List of PAIs reported to be present in genome sequences and
64 the number of G4s within PAIs.

65 **Supplementary File 1c.** Functional annotations for *E. coli* strain 1.

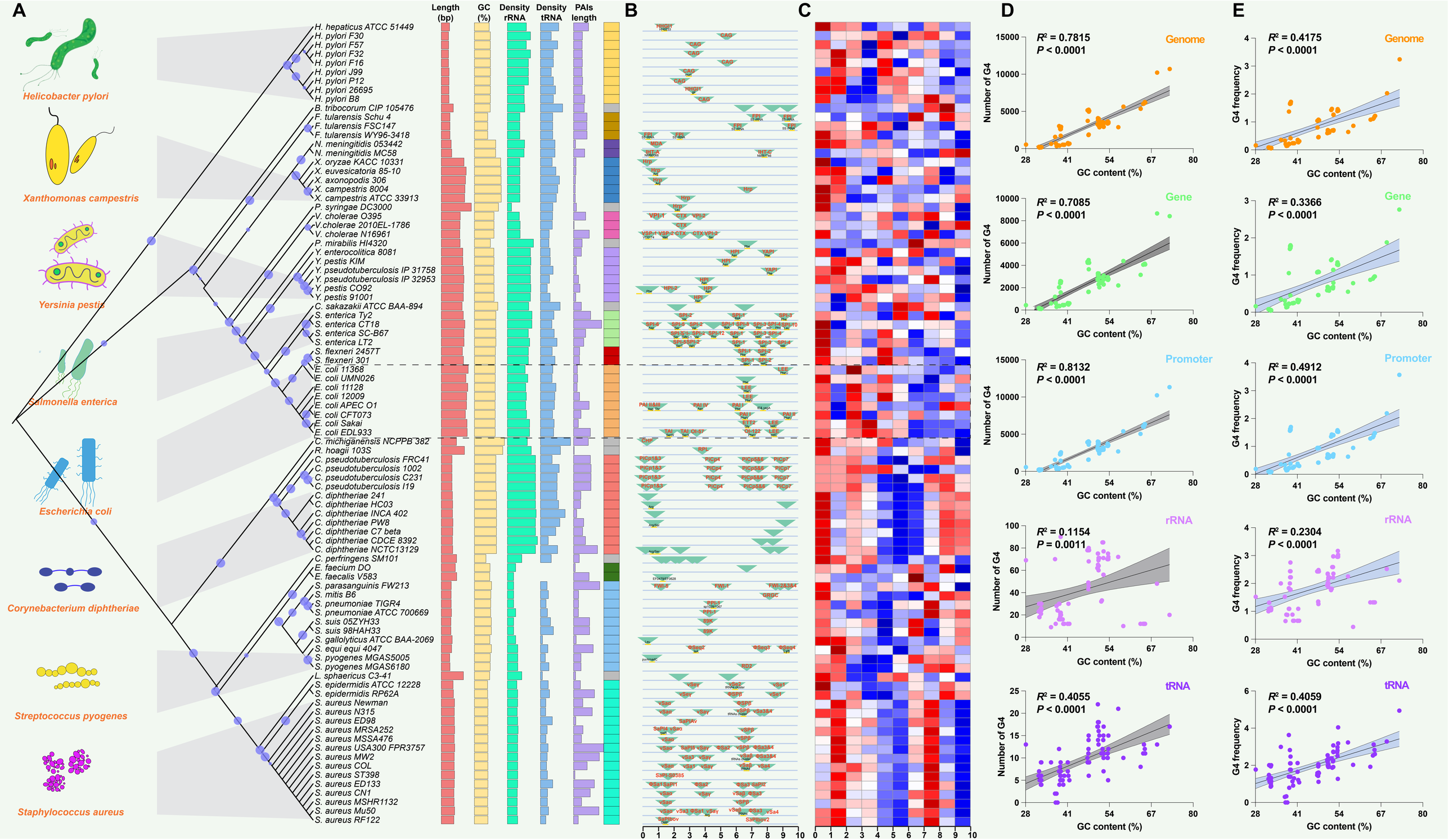
66 **Supplementary File 1d.** Functional annotations for *E. coli* strain 2.

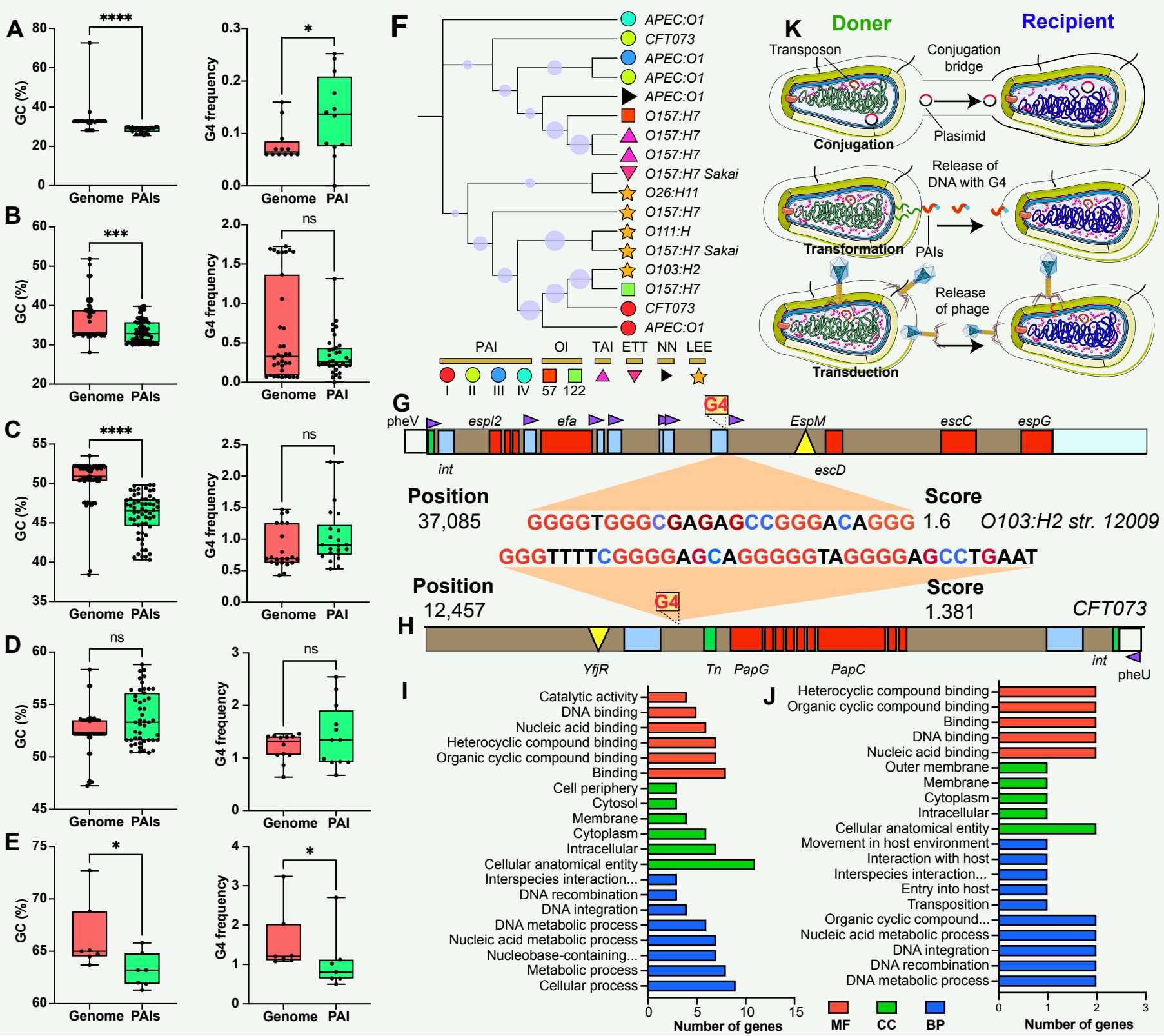

A
C
H
N
O
R
H-bond
Coordination bond
G
K+
B
C
G≥3NXG≥3NXG≥3NXG≥3
K+
D
Regulator
Ribosome
E
PAI
%G+C
70
60
50
40
30
No. nucleotides
Core genome
Repeat
tRNA
Integrase gene
Virulence gene1
Virulence gene2
Virulence gene3
Virulence gene4
IS element
Core genome



Threshold score > 1.4

Threshold score > 1.6


A
Helicobacter pylori
Xanthomonas campestris
Yersinia pestis
Salmonella enterica
Escherichia coli
Corynebacterium diphtheriae
Streptococcus pyogenes
Staphylococcus aureus
Length (bp)
GC (%)
Density rRNA
Density tRNA
PAIs length
B
C
D
E
Genome
Gene
Promoter
rRNA
tRNA
Number of G4
G4 frequency
GC content (%)
R² = 0.7815
P < 0.0001
R² = 0.4175
P < 0.0001
R² = 0.7085
P < 0.0001
R² = 0.3366
P < 0.0001
R² = 0.8132
P < 0.0001
R² = 0.4912
P < 0.0001
R² = 0.1154
P = 0.0011
R² = 0.2304
P < 0.0001
R² = 0.4055
P < 0.0001
R² = 0.4059
P < 0.0001


A
B
C
D
E
F
G
H
I
J
K
GC (%)
G4 frequency
Genome
PAIs
PAI
****
***
*
ns
APEC:O1
CFT073
O157:H7
O157:H7 Sakai
O26:H11
O111:H
O103:H2
PAI
OI
TAI
ETT
NN
LEE
I
II
III
IV
57
122
Doner
Recipient
Transposon
Conjugation bridge
Conjugation
Plasmid
Release of DNA with G4
Transformation
PAIs
Release of phage
Transduction
pheV
espI2
efa
G4
EspM
escC
espG
int
escD
Position
37,085
Score
GGGGTGGGCGAGAGCCGGGACAGGG 1.6
O103:H2 str. 12009
GGGTTTTCGGGGAGCAGGGGGTAGGGGAGCCTGAAT
Position
12,457
Score
1.381
CFT073
YfjR
Tn
PapG
PapC
int
pheU
Catalytic activity
DNA binding
Nucleic acid binding
Heterocyclic compound binding
Organic cyclic compound binding
Binding
Cell periphery
Cytosol
Membrane
Cytoplasm
Intracellular
Cellular anatomical entity
Interspecies interaction...
DNA recombination
DNA integration
DNA metabolic process
Nucleic acid metabolic process
Nucleobase-containing...
Metabolic process
Cellular process
Number of genes
Outer membrane
Movement in host environment
Interaction with host
Entry into host
Transposition
Organic cyclic compound...
MF
CC
BP