Subs Paper Things to Do:

- Or get 1st, 2nd, 3rd codon pos log regs
- ~~write dN/dS methods~~
- ~~write dN/dS results~~
- ~~write dN/dS discussion~~
- ~~write dN/dS into conclusion~~
- mol clock for my analysis?
- GC content? COG? where do these fit?

Gene Expression Paper Things to Do:

- ~~write abstract~~
- ~~write intro~~
- ~~add stuff from outline to Data section~~
- ~~create graphs for expression distribution (no sub data)~~
- ~~add # of genes to expression graphs (top)~~
- ~~average gene expression~~
- ~~write discussion~~
- ~~write conclusion~~
- ~~add into methods: filters for Hiseq, RT PCR and growth phases for data collection~~
- update supplementary figures/file

Inversions and Gene Expression Letter Things to Do:

- ~~check if opposite strand in progressiveMauve means an inversions (check visual matches the xmfa)~~
- ~~check if PARSNP and progressiveMauve both identify the same inversions (check xmfa file)~~
- create latex template for paper
- ~~put notes from papers into doc~~
- ~~use large PARSNP alignment to identify inversions~~

- confirm inversions with dot plot
- write outline for letter
- write Abstract
- write intro
- write methods
- compile tables (supplementary)
- write results
- write discussion
- write conclusion
- do same ancestral/phylogenetic analysis that I did in the subs paper

Last Week

✓look into *Streptomyces* $dN > dS$ issue

✓look into why pSymB is missing so much data in the dN/dS distribution graphs

✓send you two paper drafts, one for the substitutions paper and one for the gene expression paper

I looked into the weird points of the distribution of dN, dS, and ω across the genome and the points where dS is higher than $dN = \omega$ are real, and the high number of $dS = 0$ points in *S. meliloti* chrom is also real. We discussed this and you said to just leave everything the way it is.

As I mentioned before, the GUI for Dotter is really bad and it constantly freezes. So I am still working through how to save plots where the contrast is good enough to define the inversions.

I also continued to look into why pSymB is missing so much data, and why *Streptomyces* had $dN > dS$ for the whole genome. I realized that my definitions for the start and endings of genes was slightly off, and there was an issue with the genome positions that my program was spitting out. So I have fixed these issues and I am re-running all of this now to see if this makes a difference for the pSymB and *Streptomyces* selection issues.

I also calculated the average gene expression per replicon for fun, this is found in Table 1. *Streptomyces* is like 2 orders of magnitude lower than everything else..which is weird so I am not sure what is going on there. Do you think this is something that needs to be put into the gene expression paper?

I have also been working to put the dN, dS, and ω values for each gene into a supplementary table on github. This is slowly getting done.

I was also wondering if I should be fitting a regression to the dN, dS, and ω data to see how those three values change (if at all) with genomic position? although to me the graphs look pretty non-linear. Thoughts?

I have also started to work on my poster for SMBE and have a few questions for you about what should be included or not. I will talk to you later this week.

This Week

I hope to have the re-running of the selection and substitutions analysis done by the end of the week.

I would also like to have a dot plot for the inversions and gene expression analysis.

I also need to work on my poster for the conference.

Next Week

Continue working on poster for conference.

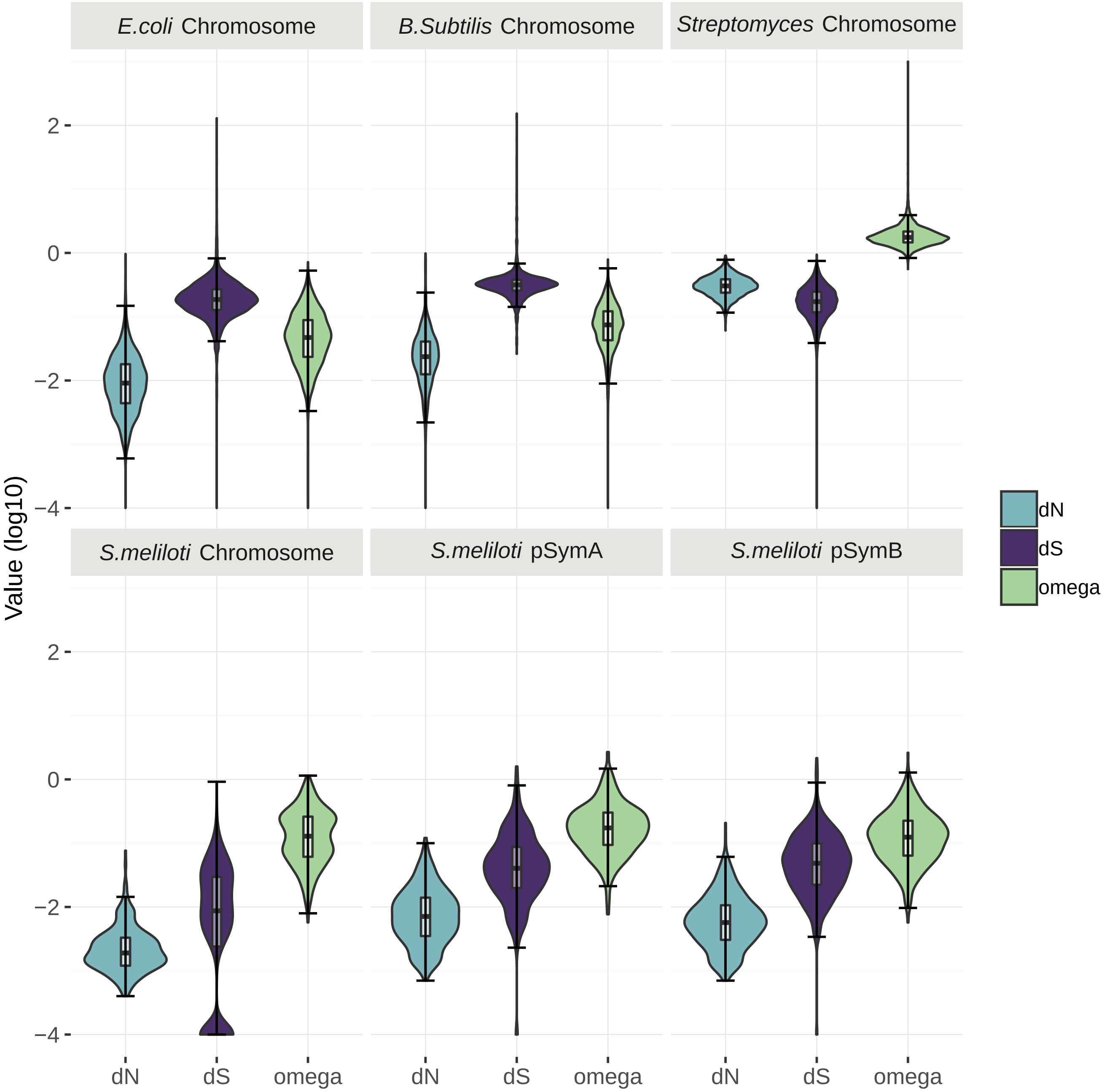
Make edits to the papers.

Move on to next steps for the inversions and gene expression analysis

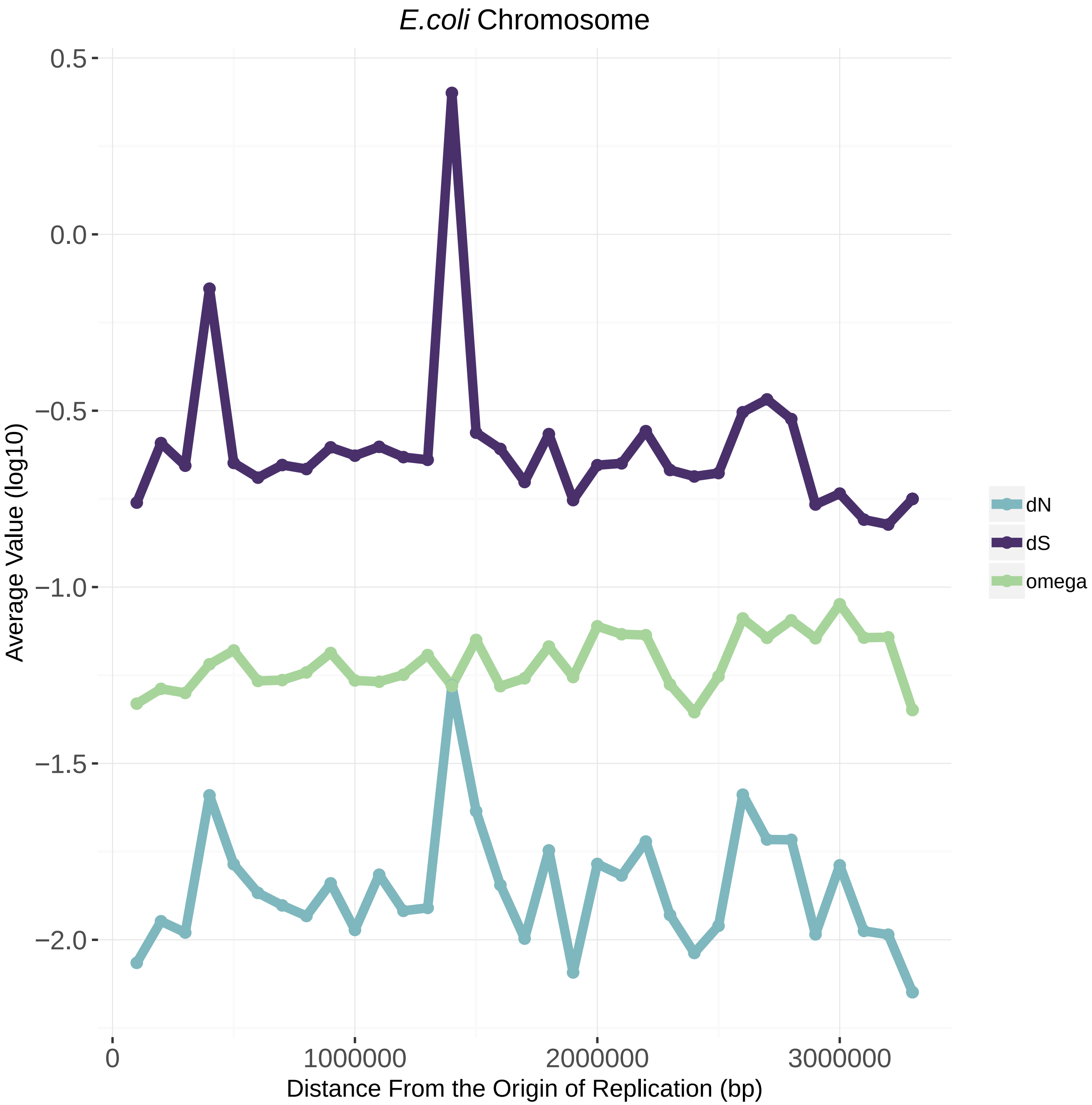
Bacteria and Replicon	Average Expression Value (CPM)
E. coli Chromosome	160.500
B. subtilis Chromosome	176.400
Streptomyces Chromosome	6.084
S. meliloti Chromosome	271.400
S. meliloti pSymA	690.100
S. meliloti pSymB	595.700

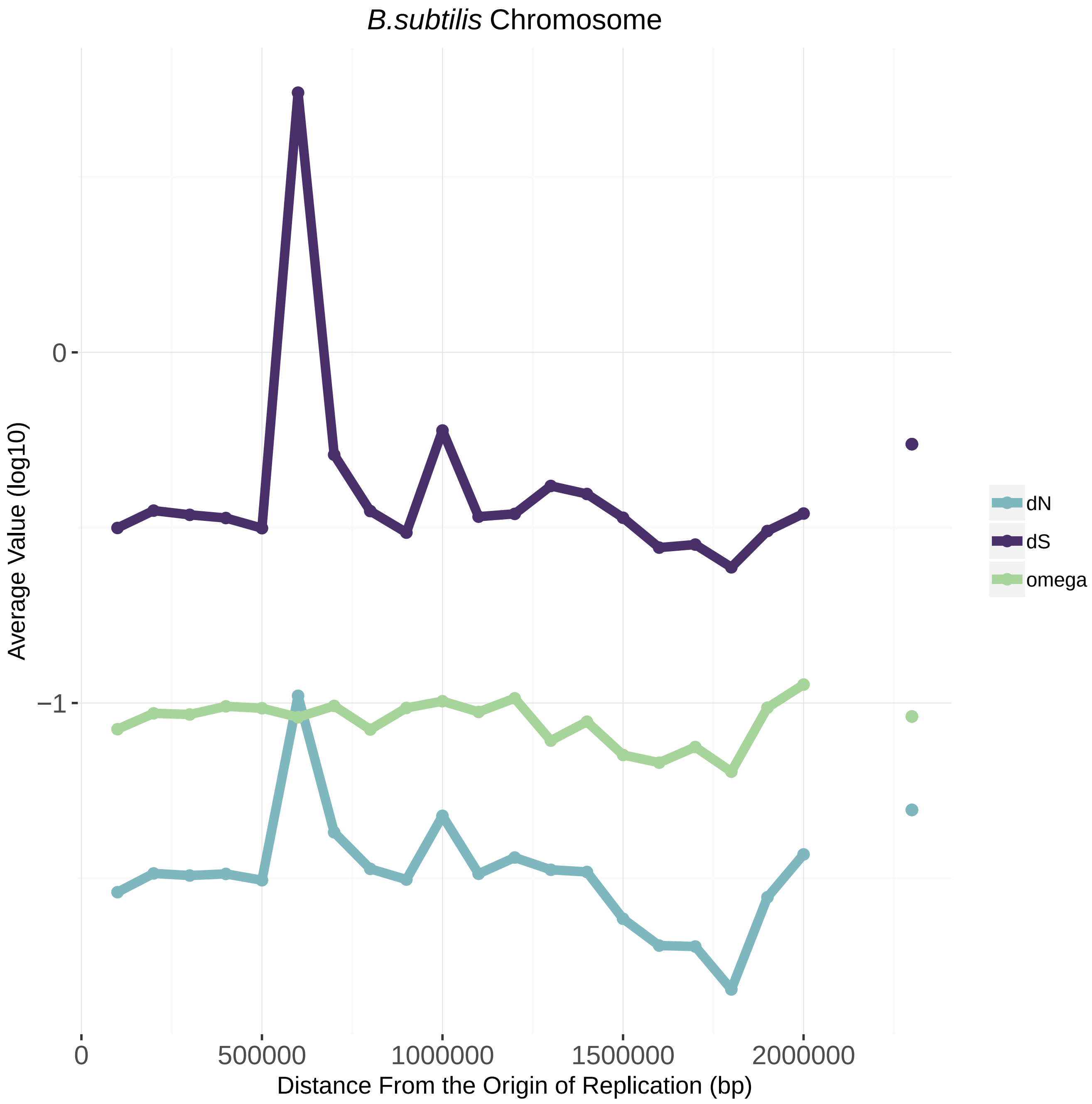
Table 1: Arithmetic gene expression calculated across all genes in each replicon. Expression values are represented in Counts Per Million.

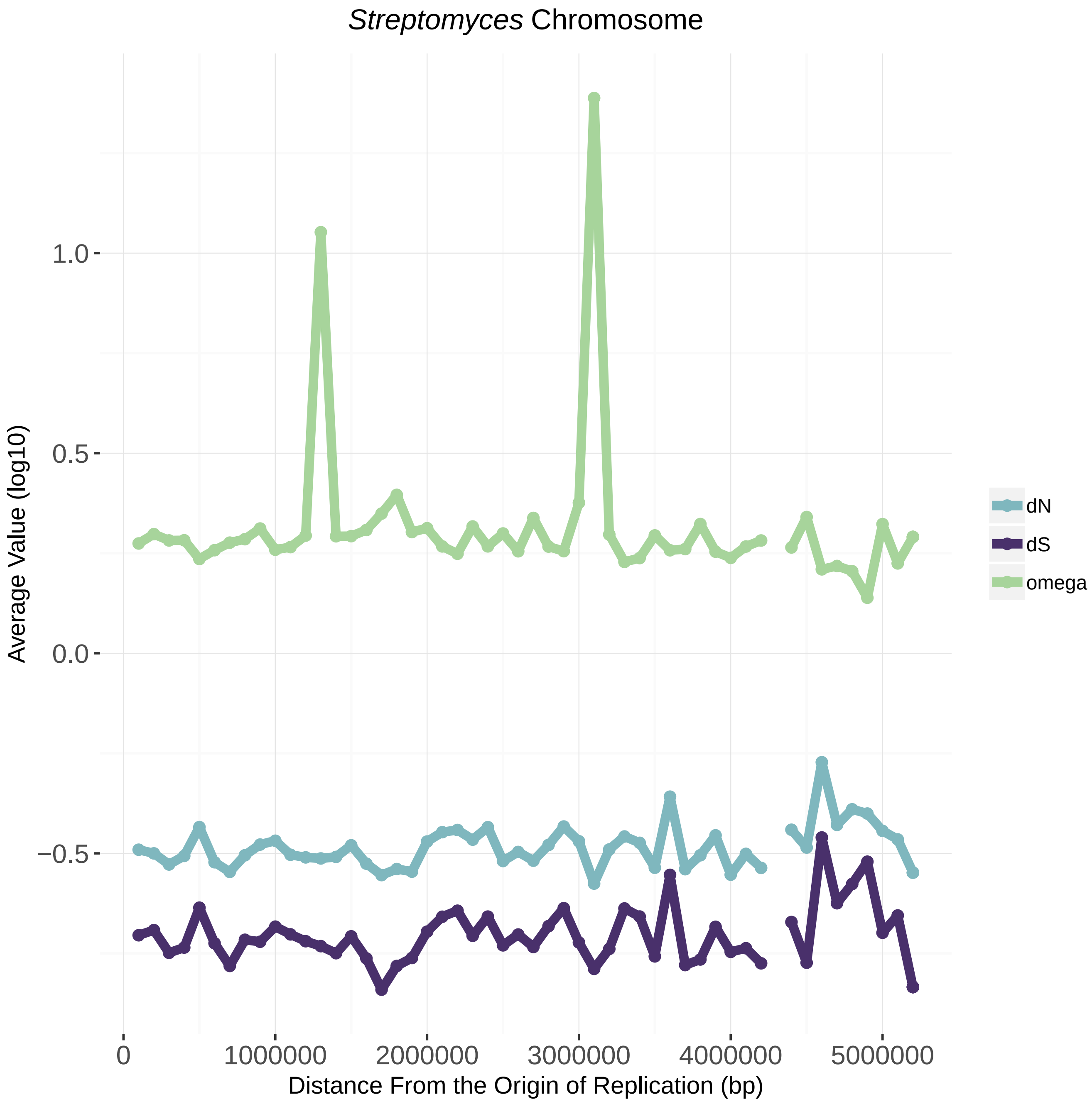
Violin plots for per gene dN, dS, and ω:

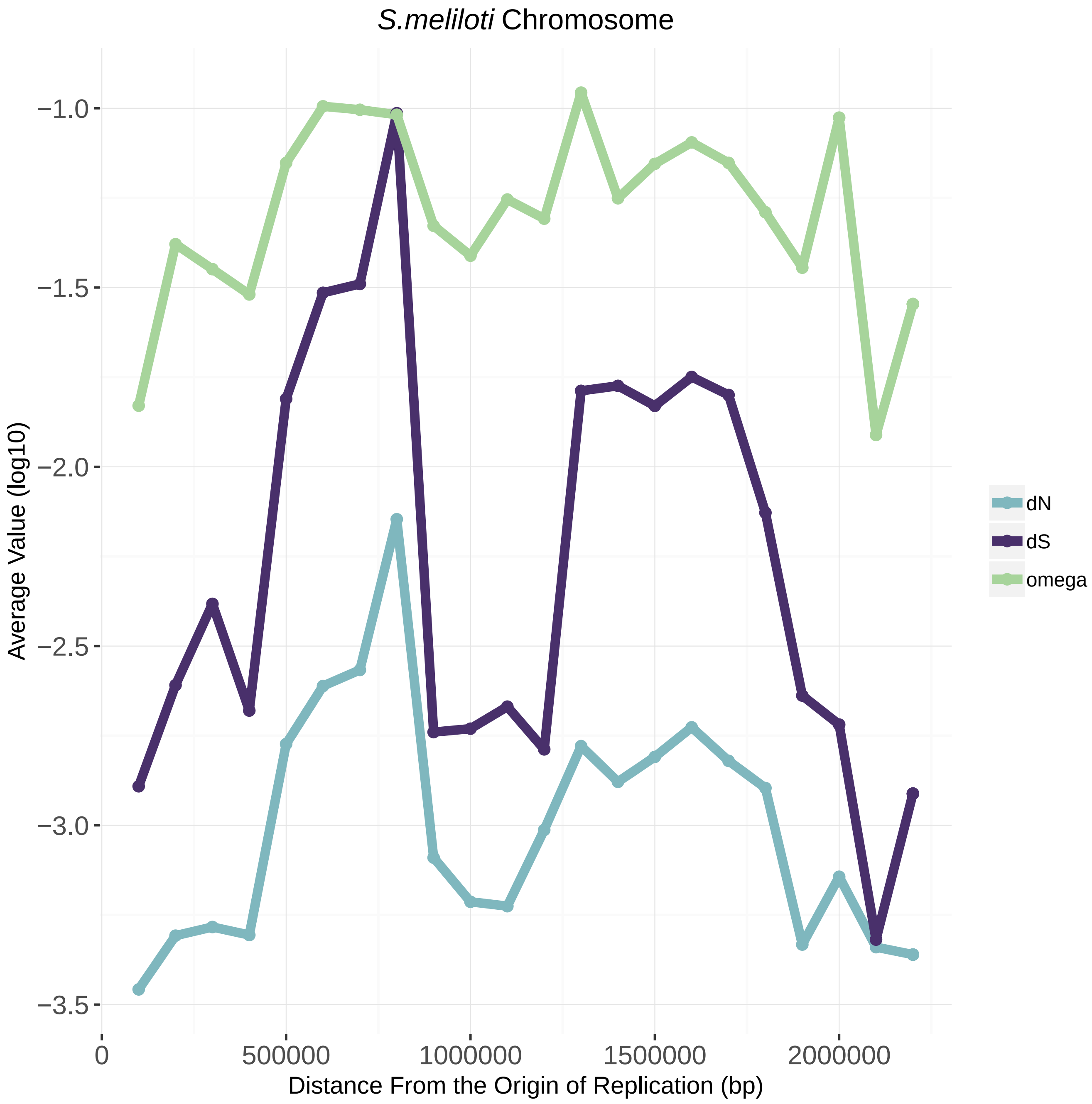


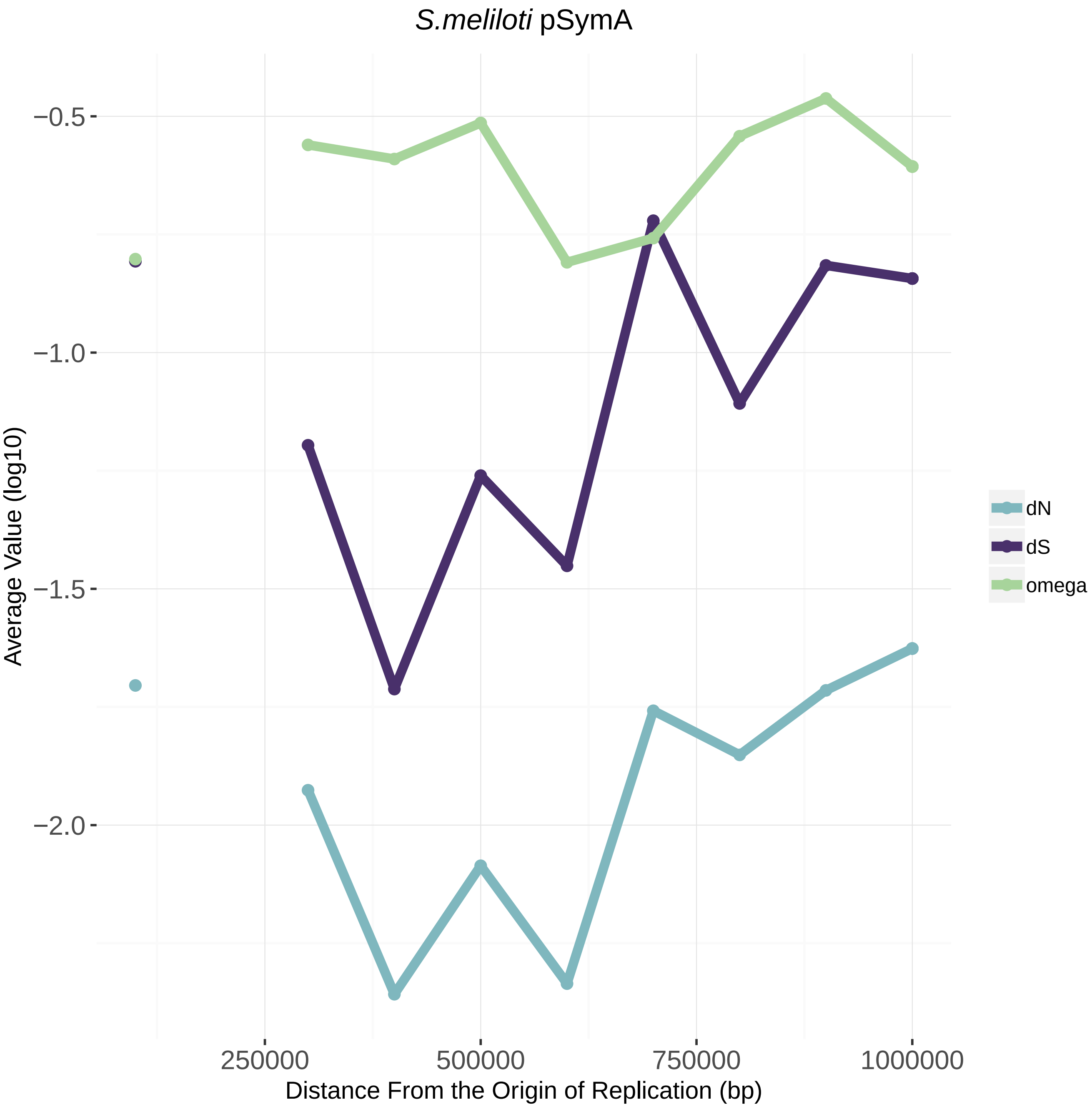
Genome Distribution for per 10kb dN, dS, and ω averages:

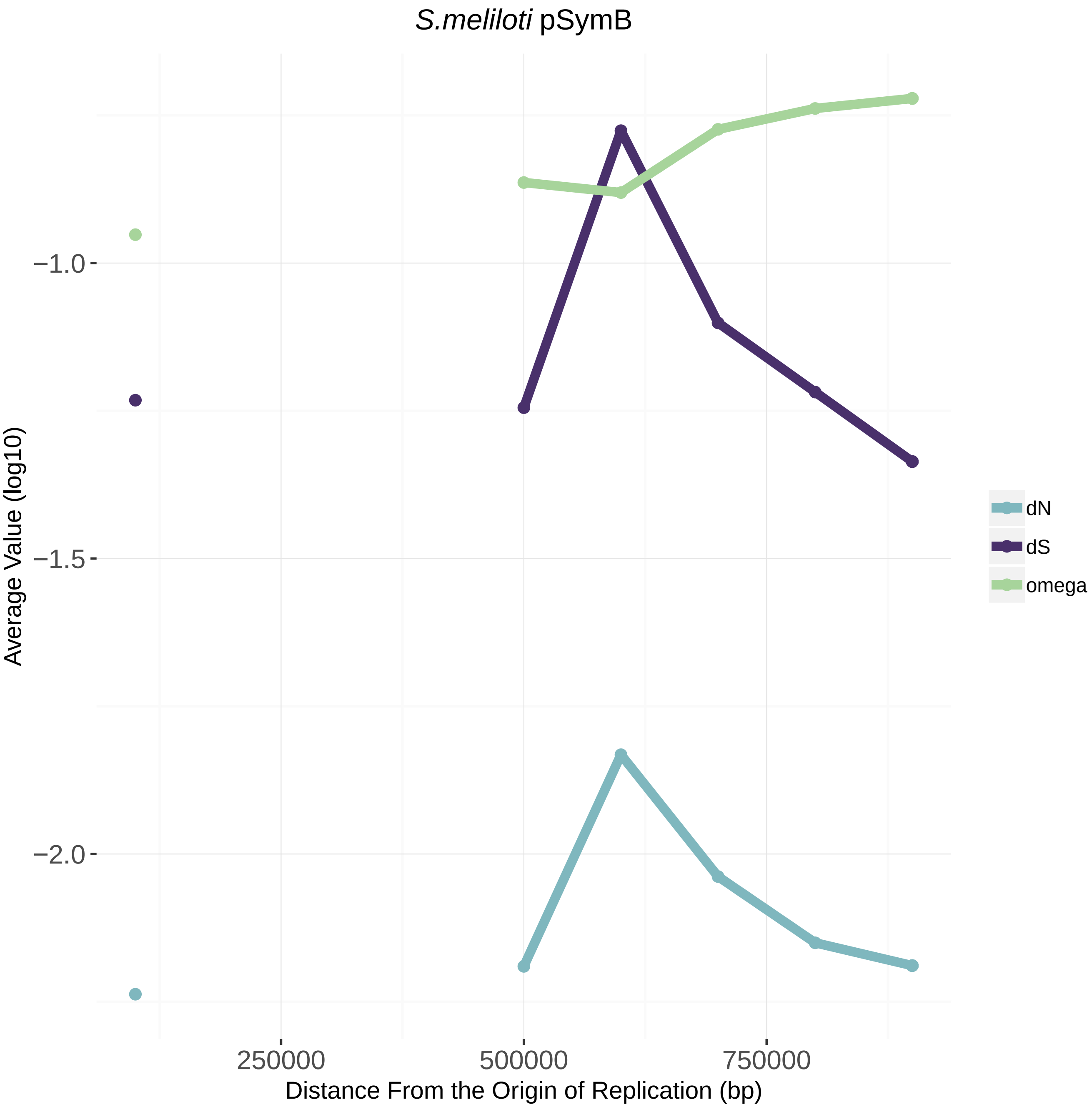

E.coli Chromosome
0.5
0.0
−0.5
−1.0
−1.5
−2.0
Average Value (log10)
0
1000000
2000000
3000000
Distance From the Origin of Replication (bp)
dN
dS
omega


B.subtilis Chromosome
Average Value (log10)
0
−1
0
500000
1000000
1500000
2000000
Distance From the Origin of Replication (bp)
dN
dS
omega


Streptomyces Chromosome
Average Value (log10)
1.0
0.5
0.0
−0.5
0
1000000
2000000
3000000
4000000
5000000
Distance From the Origin of Replication (bp)
dN
dS
omega


S.meliloti Chromosome
Average Value (log10)
−1.0
−1.5
−2.0
−2.5
−3.0
−3.5
0
500000
1000000
1500000
2000000
Distance From the Origin of Replication (bp)
dN
dS
omega


S.meliloti pSymA
−0.5
−1.0
−1.5
−2.0
Average Value (log10)
250000
500000
750000
1000000
Distance From the Origin of Replication (bp)
dN
dS
omega


S.meliloti pSymB
Average Value (log10)
−1.0
−1.5
−2.0
250000
500000
750000
Distance From the Origin of Replication (bp)
dN
dS
omega

	Gene Average			Genome Average		
Bacteria and Replicon	dS	dN	ω	dS	dN	ω
E. coli Chromosome	0.2924	0.0144	0.0604	0.2600	0.0133	0.0556
B. subtilis Chromosome	0.6526	0.0358	0.0891	0.5267	0.0321	0.0828
Streptomyces Chromosome	0.1924	0.3201	2.6404	0.1775	0.3017	2.4358
S. meliloti Chromosome	0.0134	0.0014	0.0844	0.0134	0.0013	0.0930
S. meliloti pSymA	0.0798	0.0109	0.2320	0.0800	0.0103	0.2218
S. meliloti pSymB	0.0814	0.0086	0.1639	0.0782	0.0082	0.1590

Table 2: Weighted averages calculated for each bacterial replicon on a per genome basis using the gene length as the weight. Arithmetic mean calculated for the per gene averages for each bacterial replicon.

Bacteria and Replicon	Average Replicon Length	# of Coding Sites	# of Non-Coding Sites	# of Subs Coding	# of Subs Non-Coding
E. coli Chromosome	5082529	2960007	191748	207199	9534
B. subtilis Chromosome	4077077	2074653	102906	205150	6187
Streptomyces Chromosome	8497577	2422980	21581	551530	3670
S. meliloti Chromosome	3426881	1931139	199425	6684	842
S. meliloti pSymA	1455940	419223	34213	9832	943
S. meliloti pSymB	1664597	552816	22098	11699	645

Table 3: Total proportion of coding and non-coding sites in each replicon and the percentage of those sites that have a substitution (multiple substitutions at one site are counted as two substitutions).

Bacteria and Replicon	Coding Sequences	Non-Coding Sequences
E. coli Chromosome	$-9.983\times10^{-8***}$	$6.994\times10^{-8***}$
B. subtilis Chromosome	$-1.071\times10^{-7***}$	$-9.861\times10^{8***}$
Streptomyces Chromosome	$-2.626\times10^{-8***}$	$3.615\times10^{-7***}$
S. meliloti Chromosome	$-1.367\times10^{-7***}$	$-1.510\times10^{-7*}$
S. meliloti pSymA	$-1.075\times10^{-7*}$	NS
S. meliloti pSymB	$2.878\times10^{-7***}$	$8.595\times10^{-7***}$

Table 4: Logistic regression analysis of the number of substitutions along all positions of the genome of the respective bacteria replicons. These genomic positions were split up into the coding and non-coding regions of the genome. Grey coloured boxes indicate a negative logistic regression coefficient estimate. All results are statistically significant. Logistic regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication. All results are marked with significance codes as followed: < 0.001 = '***', $0.001 < 0.01$ = '**', $0.01 < 0.05$ = '*', > 0.05 = 'NS'.

Bacteria Strain/Species	GEO Accession Number	Date Accessed
E. coli K12 MG1655	GSE60522	December 20, 2017
E. coli K12 MG1655	GSE73673	December 19, 2017
E. coli K12 MG1655	GSE85914	December 19, 2017
E. coli K12 MG1655	GSE40313	November 21, 2018
E. coli K12 MG1655	GSE114917	November 22, 2018
E. coli K12 MG1655	GSE54199	November 26, 2018
E. coli K12 DH10B	GSE98890	December 19, 2017
E. coli BW25113	GSE73673	December 19, 2017
E. coli BW25113	GSE85914	December 19, 2017
E. coli O157:H7	GSE46120	August 28, 2018
E. coli ATCC 25922	GSE94978	November 23, 2018
B. subtilis 168	GSE104816	December 14, 2017
B. subtilis 168	GSE67058	December 16, 2017
B. subtilis 168	GSE93894	December 15, 2017
B. subtilis 168	GSE80786	November 16, 2018
S. coelicolor A3	GSE57268	March 16, 2018
S. natalensis HW-2	GSE112559	November 15, 2018
S. meliloti 1021 Chromosome	GSE69880	December 12, 2017
S. meliloti 2011 pSymA	NC_020527 (Dr. Finan)	April 4, 2018
S. meliloti 1021 pSymA	GSE69880	November 15, 18
S. meliloti 2011 pSymB	NC_020560 (Dr. Finan)	April 4, 2018
S. meliloti 1021 pSymB	GSE69880	November 15, 18

Table 5: Summary of strains and species found for each gene expression analysis. Gene Expression Omnibus accession numbers and date accessed are provided.

Bacteria and Replicon	Coefficient Estimate	Standard Error	P-value
E. coli Chromosome	-6.03×10^{-5}	1.28×10^{-5}	2.8×10^{-6}
B. subtilis Chromosome	-9.7×10^{-5}	2.0×10^{-5}	1.2×10^{-6}
Streptomyces Chromosome	-1.17×10^{-6}	1.04×10^{-7}	$<2\times10^{-16}$
S. meliloti Chromosome	3.97×10^{-5}	4.25×10^{-5}	NS (3.5×10^{-1})
S. meliloti pSymA	1.39×10^{-3}	2.53×10^{-4}	4.9×10^{-8}
S. meliloti pSymB	1.46×10^{-4}	2.03×10^{-4}	NS ($5.34.7\times10^{-1}$)

Table 6: Linear regression analysis of the median counts per million expression data along the genome of the respective bacteria replicons. Linear regression was calculated after the origin of replication was moved to the beginning of the genome and all subsequent positions were scaled around the origin accounting for bidirectionality of replication.