Subs Paper Things to Do:

- ~~write dN/dS methods~~
- ~~write dN/dS results~~
- ~~write dN/dS discussion~~
- ~~write dN/dS into conclusion~~
- ~~spatial analysis of dN, dS, and ω~~
- 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

Last week I finally fixed the previous issue where pseudogenes and genes with "joins" in them were messing up my code. With this, I decided to more accurately describe the coding and non-coding sections of the genome. All coding regions will now be referred to as protein coding regions, and non-coding regions will be referred to as non-protein coding regions. So all intergenic, RNA, and pseudogenes are classified as non-coding regions. I started to re-run the selection and substitution analysis on Thursday. *Streptomyces* takes a really really long time to run because it has the most blocks (and the biggest genome) so it is still running through the both analysis. All the other bacteria are done with the substitutions analysis and the graphs and results can be found below.

I hit a small snag in the selection analysis, it looks like the alignments that are outputted from my program do not all have a multiple of 3 as its length. I am currently looking into this. Hopefully I can solve this issue by today and have the results for the selection analysis by the end of the week.

I also wanted your opinion about something that was mentioned at the conference. Someone asked me if I was including RNA in my "coding" sections? Because RNA often is under different selective pressures than coding or other "non-coding" sections, they suggested that I do my analysis on just all RNA to see if there is any sort of trend with respect to distance from the origin. I was wondering what you think about this and if you think it is worth it for me to do?

I have begun compiling my committee report for Aug 26th. I am hoping to send it out to everyone by Monday Aug 19th, so there is time to read it before the meeting.

This Week

This week I need to figure out why there are non-multiple of 3 genes being outputted by my analysis (which should not be happening). Once this is complete, I can continue to re-run the selection analysis.

I would like to finish my committee report by the end of the weekend.

While all that is running, I need to keep working on the inversions and gene expression stuff.

Next Week

Assuming the re-running of my analysis is complete, I would like to work intensely on the inversions and gene expression stuff and get a good chunk of that started.

Bacteria and Replicon	Coding Sequences	Non-Coding Sequences
E. coli Chromosome	-1.313×10^{-7}***	NS
B. subtilis Chromosome	-6.732×10^{-8}***	NS
Streptomyces Chromosome		
S. meliloti Chromosome	-9.185×10^{-8}***	-1.718×10^{-7}***
S. meliloti pSymA	-8.121×10^{-7}***	-1.247×10^{-7}***
S. meliloti pSymB	1.655×10^{-7}***	4.105×10^{-7}***

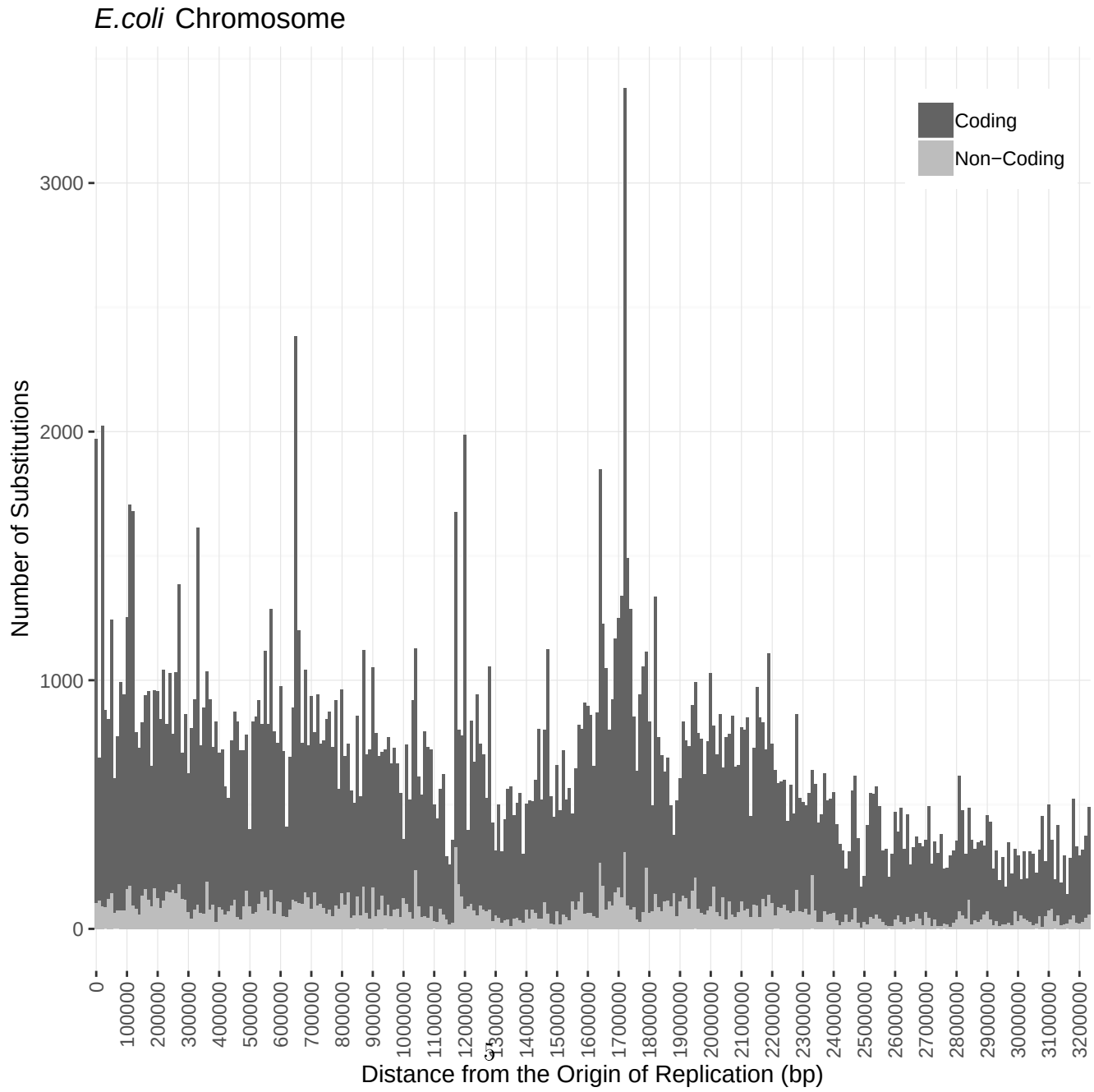
Table 1: Logistic regression analysis of the number of substitutions along all positions of the genome of the respective bacteria replicons. These genomic positions were split up into the coding and non-coding regions of the genome. Grey coloured boxes indicate a negative logistic regression coefficient 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'.

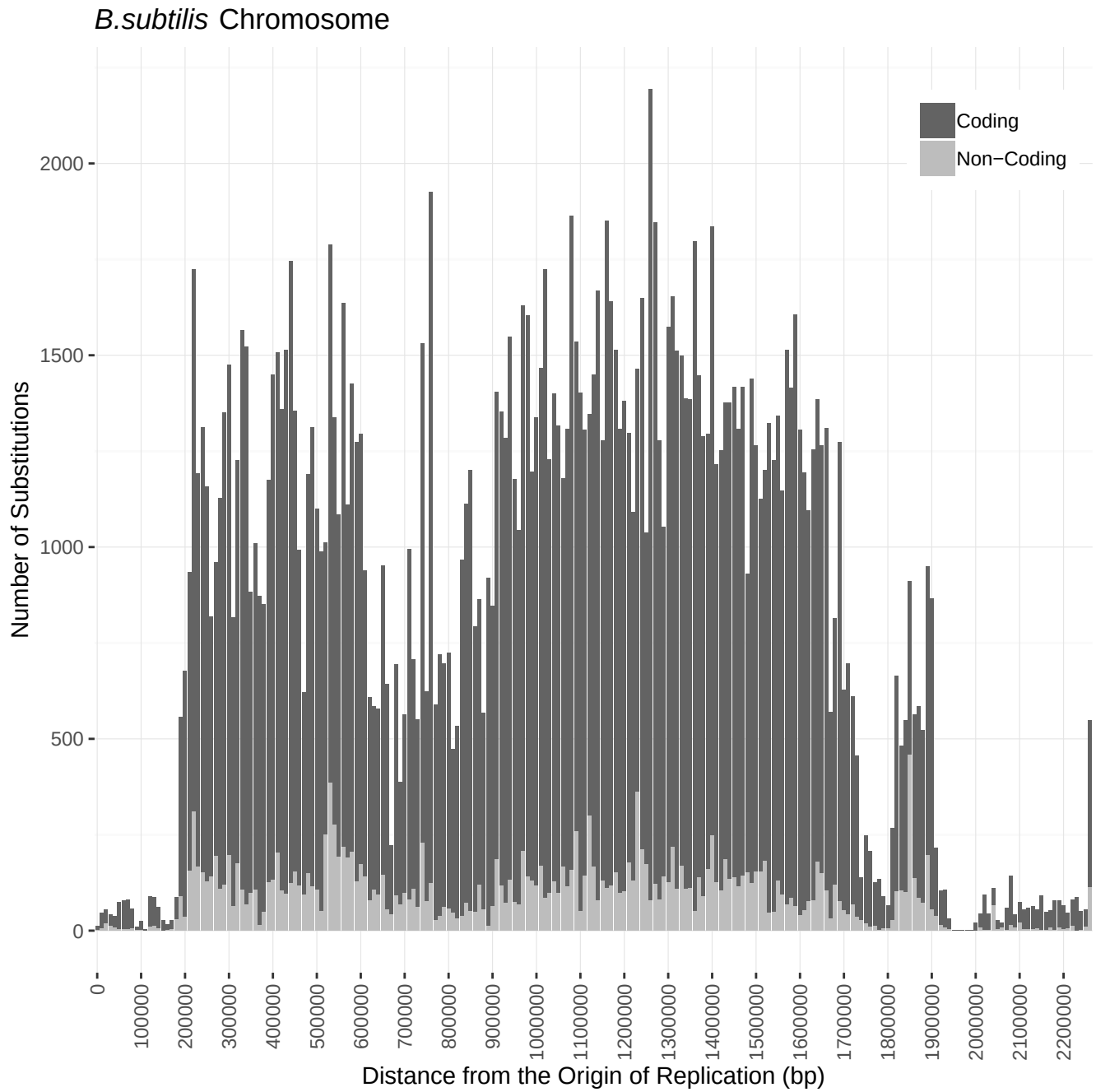
Bacteria and Replicon	Average Expression Value (CPM)
E. coli Chromosome	160.500
B. subtilis Chromosome	176.400
Streptomyces Chromosome	6.084
S. meliloti Chromosome	271.400
S. meliloti pSymA	690.100
S. meliloti pSymB	595.700

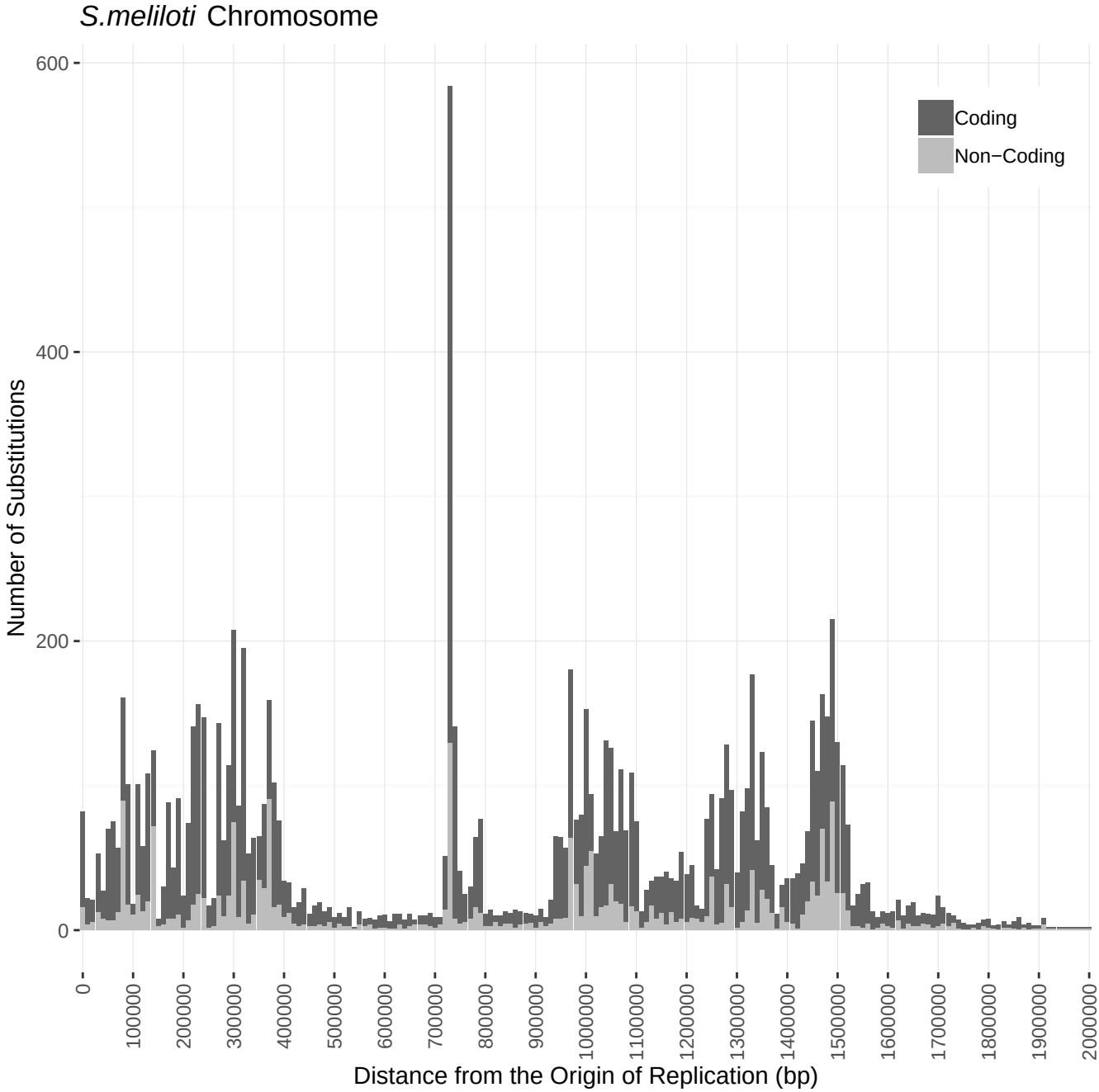
Table 2: Arithmetic gene expression calculated across all genes in each replicon. Expression values are represented in Counts Per Million.

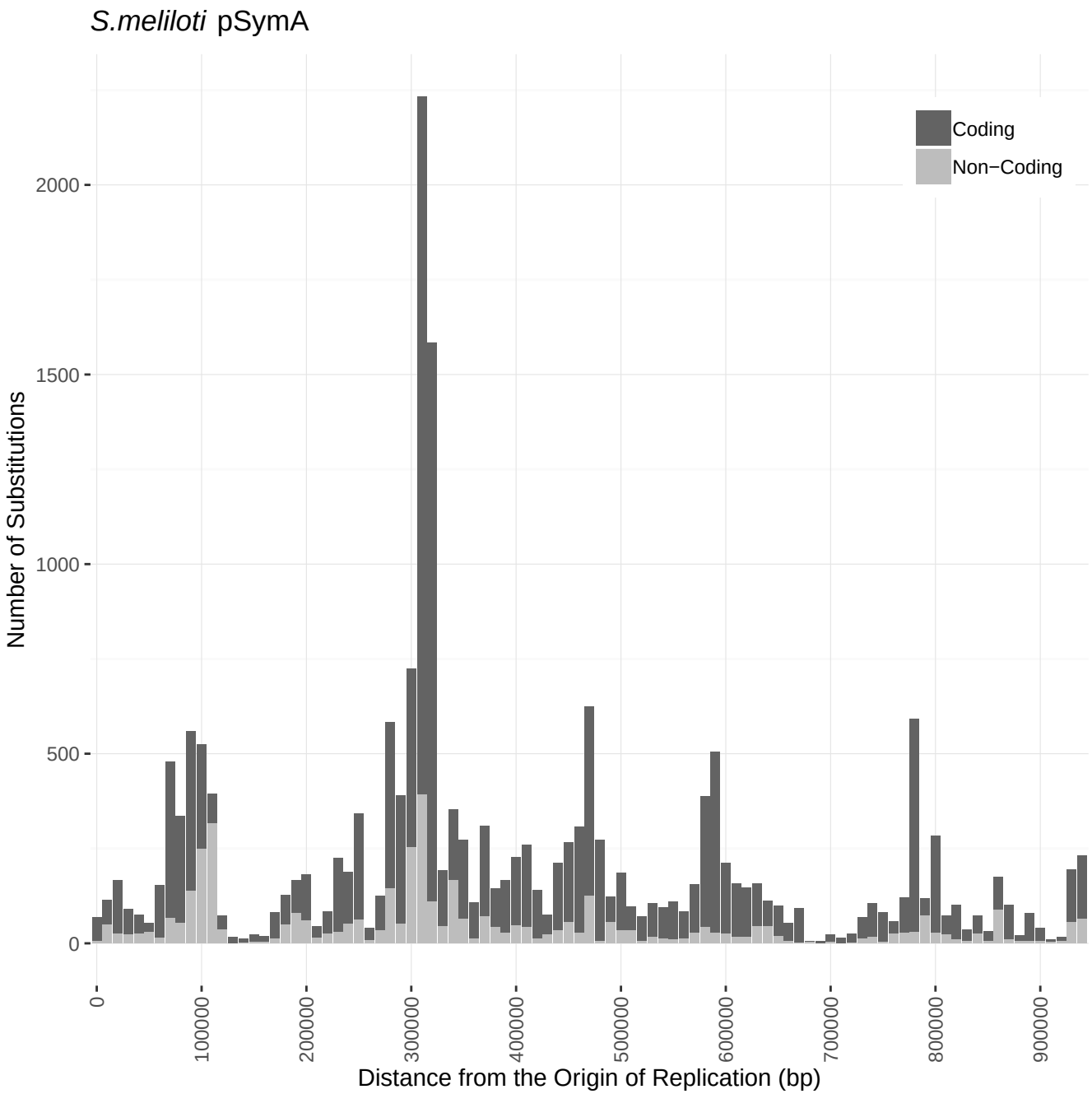
Bacteria and Replicon	Gene Average			Genome Average		
	dS	dN	ω	dS	dN	ω
E. coli Chromosome	1.0468	0.1330	1.3183	0.6491	0.0364	0.2432
B. subtilis Chromosome	4.652	0.2333	2.4200	1.0879	0.0703	0.3852
Streptomyces Chromosome						
S. meliloti Chromosome	0.0184	0.0012	0.1069	0.0187	0.0013	0.0962
S. meliloti pSymA	1.0602	0.7451	5.1290	0.4100	0.0863	0.8311
S. meliloti pSymB	3.2602	0.0256	0.3878	0.1436	0.0100	0.1943

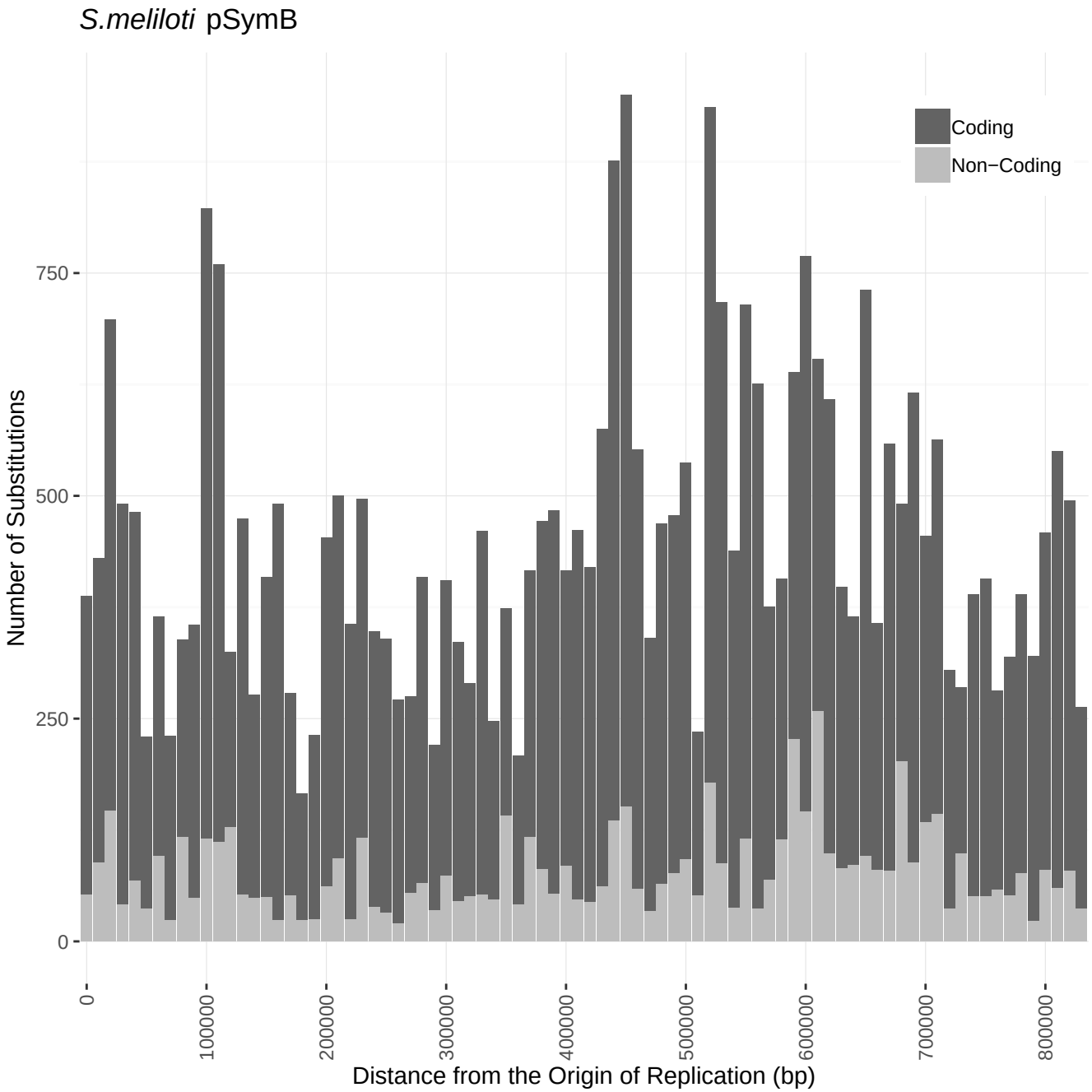
Table 3: Weighted averages calculated for each bacterial replicon on a per genome basis using the gene length as the weight. Arithmetic mean calculated for the per gene averages for each bacterial replicon.




B.subtilis Chromosome
Coding
Non−Coding
Number of Substitutions
0
500
1000
1500
2000
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000
1300000
1400000
1500000
1600000
1700000
1800000
1900000
2000000
2100000
2200000
Distance from the Origin of Replication (bp)


S.meliloti Chromosome
Coding
Non−Coding
Number of Substitutions
0
200
400
600
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
1000000
1100000
1200000
1300000
1400000
1500000
1600000
1700000
1800000
1900000
2000000
Distance from the Origin of Replication (bp)


S.meliloti pSymA
Coding
Non−Coding
Number of Substitutions
0
500
1000
1500
2000
0
100000
200000
300000
400000
500000
600000
700000
800000
900000
Distance from the Origin of Replication (bp)


S.meliloti pSymB
Coding
Non−Coding
Number of Substitutions
0
250
500
750
0
100000
200000
300000
400000
500000
600000
700000
800000
Distance from the Origin of Replication (bp)

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 4: 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 5: 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.