

1 Are EnTrI results biased?

Since transposon insertion biases can affect the essentiality level inferred from transposon mutagenesis experiments, the dataset has been tested for two types of biases: the distance from the origin and GC content. The distance bias in every individual strain is depicted in Figure 1. These plots indicate that the bias is negligible in some strains like *Salmonella typhi*, while the insertion indices of the genes in other strains need to be normalised by their distances from the origin. We have used the LOESS curve and for each strain, divided the insertion indices by the predicted LOESS value to normalise the insertion indices. As the distribution of new insertion indices will be around 1, we have multiplied the resulted values by the mean of the initial insertion indices to have a distribution around the mean. The results are shown in Figure 2.

We have also checked for GC bias. There was a bias towards the GC content of the genes and we normalised the insertion indices for GC content in the same way as we did for gene position. The results, before and after the normalisation, are plotted in Figure 3. To see if there is any positional bias for any nucleotide, the nucleotides around the insertion sites (the insertion site and 10 nucleotides on each side) are stacked on top of each other and a sequence logo is generated from these sequences. It can be inferred from Figure 4 that there is no significant bias in any position.

2 Can we recover phylogenetic information from the essential genes?

To get the evolutionary relationship among all our strains, we collected all clusters with one and only one gene per genome and concatenated all the genes corresponding to every strain. Then aligned them using mafft and generated a phylogenetic tree using fasttree software. The resulted phylogenetic tree is depicted in Figure 5.

To test if the same tree can be obtained from the essentiality of genes, we have selected all clusters that contain exactly one gene from each strain (82 clusters) and made a binary matrix from the es-

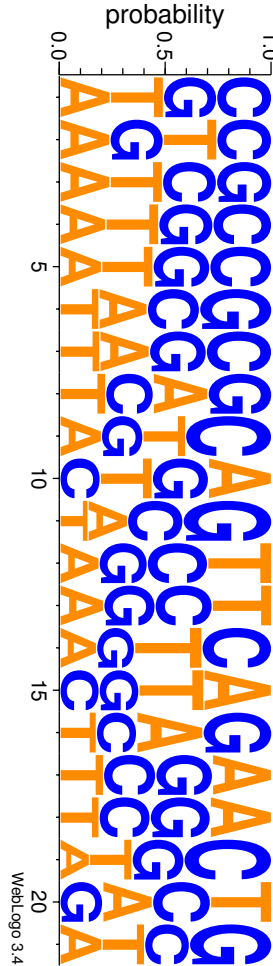


Figure 4: The nucleotides around the insertion sites (the insertion site and 10 nucleotides on each side) are stacked on top of each other and a sequence logo is generated from these sequences using webLogo stand-alone package. The height of the letter stack in each position shows how conserved the bases in that position are.

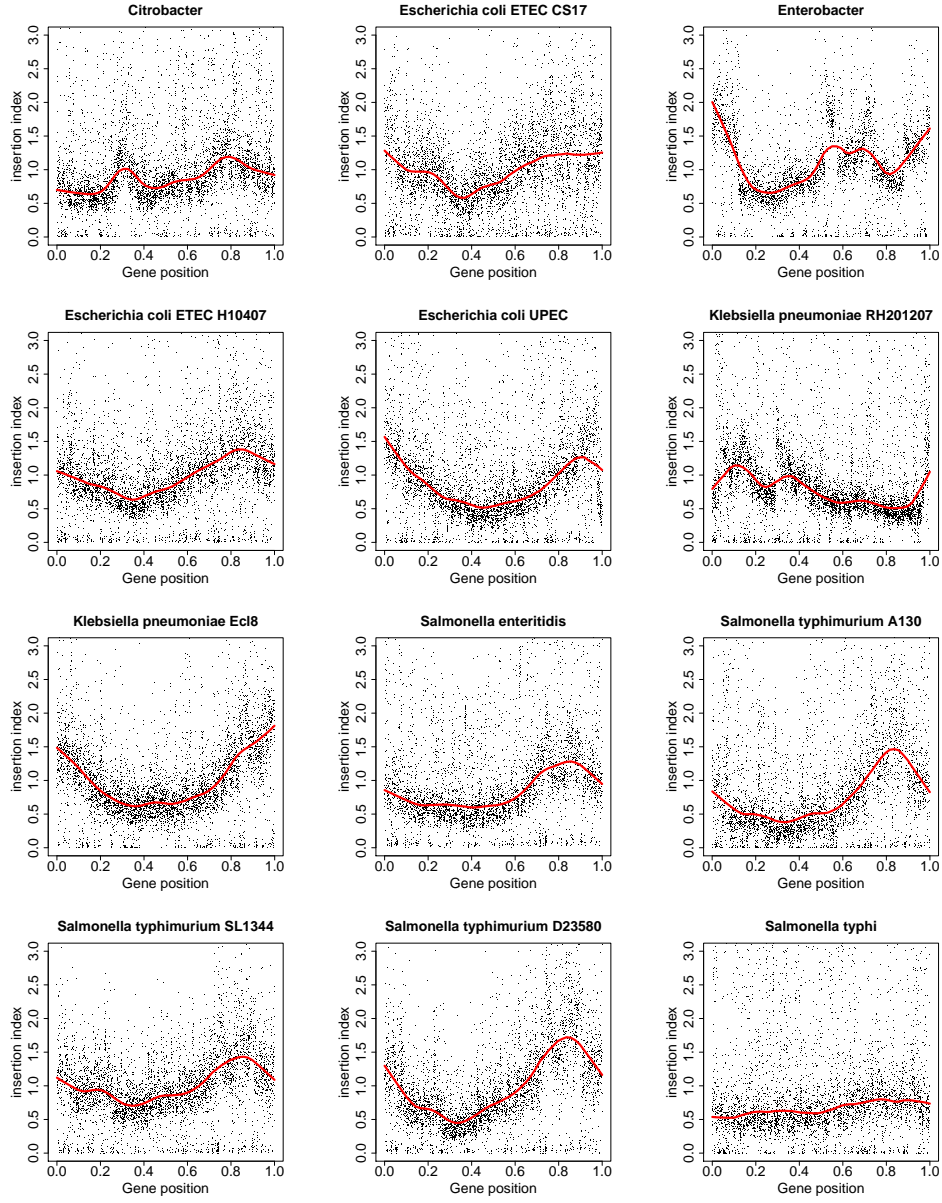


Figure 1: The bias towards the position of the gene for every individual strain. The red curves show the fitted LOESS curves.

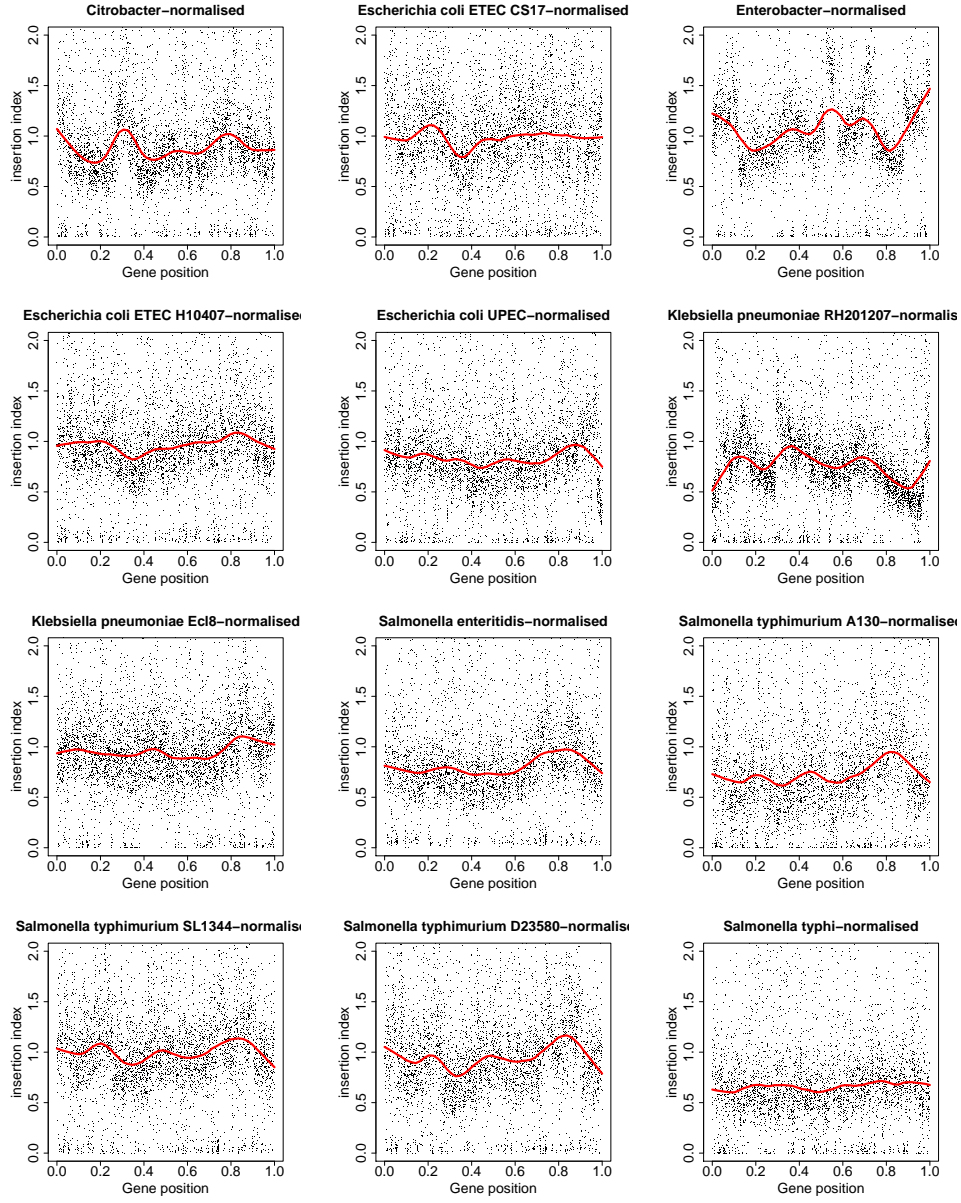


Figure 2: The insertion indices are normalised by the predicted LOESS value and the mean of the insertion indices. The red curves show the fitted LOESS curves.

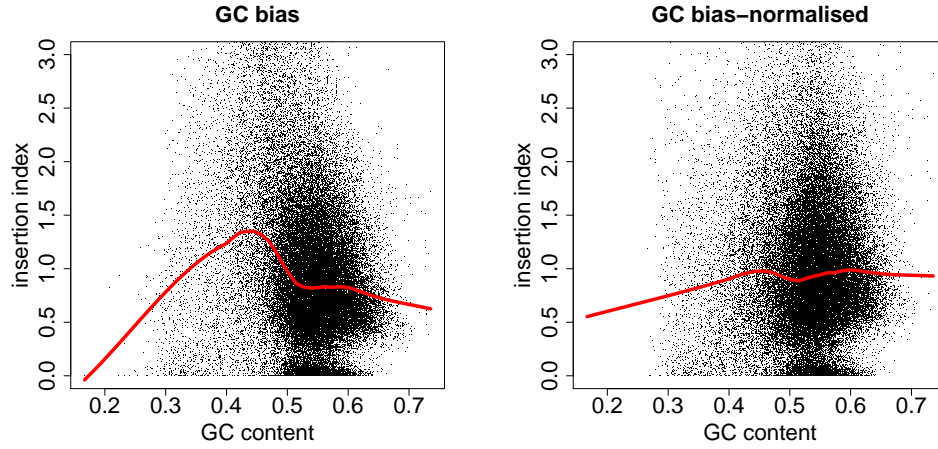


Figure 3: The bias towards GC content. The LOESS curve is shown in red.

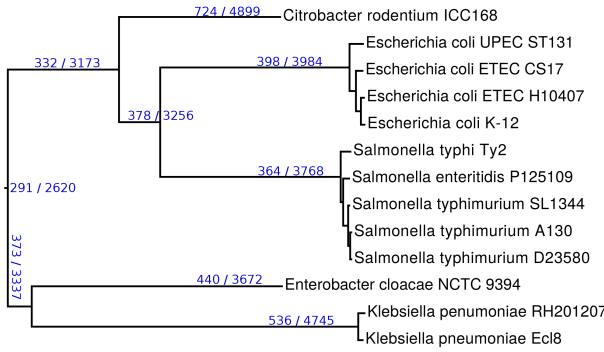


Figure 5: The tree is generated from all the clusters with one and only one gene per strain.

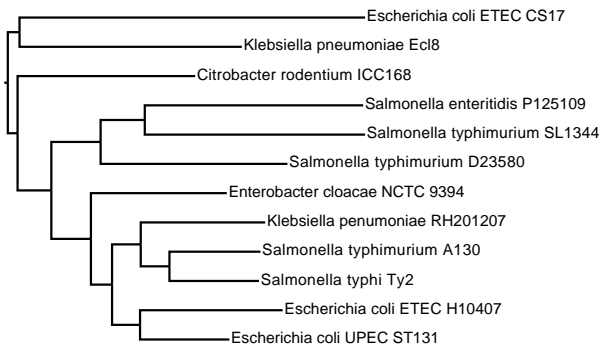


Figure 6: The phylogenetic tree is generated using “phylip neighbor” software.

sententiality of the genes in these clusters. If a gene is essential in a strain, the corresponding value in the matrix is 1 and if the gene is not essential, the value is 0. Then, we have generated a distance matrix from these values using Bray-Curtis distance and plotted a phylogenetic tree. Figure 6 indicates that the resulting tree does not maintain the phylogenetic information of the species under study.

We have also compared every pair of strains and calculated the number of genes that are essential in one strain and absent in the other, the number of genes that are essential in both strains, the number of genes that are essential in one strain and present but not essential in the other strain, the number of genes that are present in one strain and absent in the other, and the number of genes that are shared

between the two strains. The resulted heatmaps can be seen in Figure 7. The dendrograms obtained from heatmaps in this figure are consistent with the species tree to some extent.

3 Are conserved genes more likely to be essential?

To find out whether conserved genes are more likely to be essential or not we have counted the number of essential, sometimes essential and never essential genes in both core and accessory genomes. The results are summarised in Table 1. Core genes are the ones that have at least one copy per genome, otherwise, the genes are called accessory.

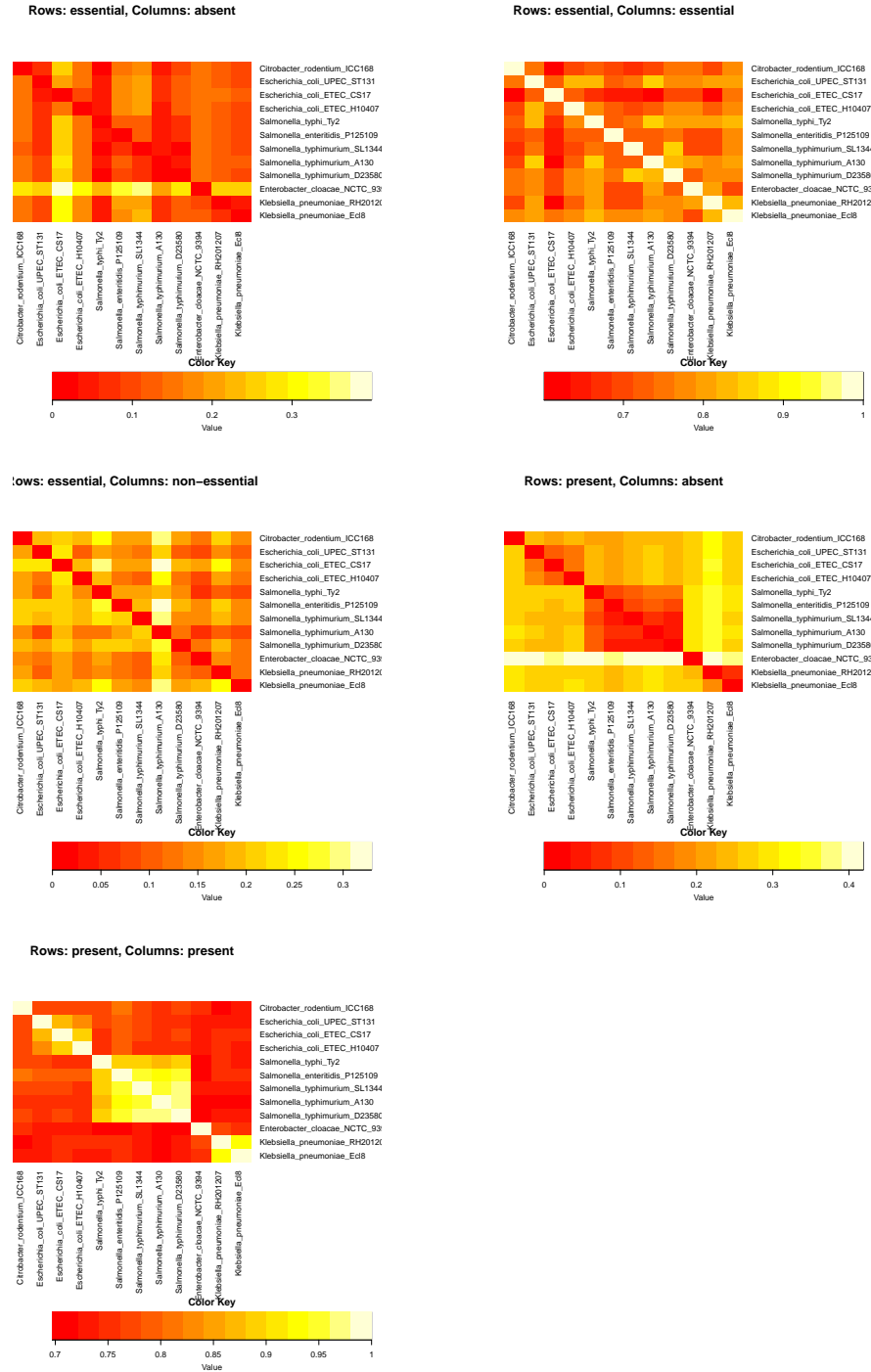


Figure 7: The number of genes that are essential in one strain and absent in the other (upper left), the number of genes that are essential in both strains (upper right), the number of genes that are essential in one strain and present but not essential in the other strain (middle left), the number of genes that are present in one strain and absent in the other (middle right), and the number of genes that are shared between the two strains (lower left).

Species Name	Core				Accessory			
	Ess	SEss	NEss	Sum	Ess	SEss	NEss	Sum
Salmonella typhimurium A130	266	665	1621	2552	53	327	1528	1908
Salmonella typhimurium D23580	267	670	1624	2561	54	339	1550	1943
Salmonella typhimurium SL1344	266	674	1630	2570	63	350	1546	1959
Salmonella enteritidis P125109	266	679	1615	2560	61	311	1015	1387
Salmonella typhi Ty2	265	628	1564	2457	56	294	1505	1855
Escherichia coli UPEC ST131	299	685	1635	2619	62	323	1767	2152
Escherichia coli ETEC CS17	280	656	1603	2539	107	356	1586	2049
Escherichia coli ETEC H10407	284	656	1623	2563	89	329	1743	2161
Escherichia coli K-12	279	657	1612	2548	174	278	1311	1763
Citrobacter rodentium ICC168	304	685	1630	2619	96	356	1868	2320
Enterobacter cloacae NCTC 9394	280	623	1526	2429	34	129	1128	1291
Klebsiella pneumoniae RH201207	357	864	1780	3001	69	279	2314	2662
Klebsiella pneumoniae Ecl8	357	840	1770	2967	61	225	1751	2037

Table 1: The number of essential (Ess), sometimes essential (SEss) and never essential (NEss) genes in both core and accessory genomes. Core genes are the ones that have at least one copy per genome, otherwise, the genes are called accessory.