Is essentiality of genes conserved in Enterobacteriaceae?

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Submitted to Proceedings of the National Academy of Sciences of the United States of America

essentiality | conservation | transposon insertion

Introduction

Studying the essentiality of genes helps with identifying the fundamental processes necessary for cell viability [1]. So far, scientists have studied the essential genes in organisms from different domains of life [2]. The results have led to new insights for developing new antibiotics that target essential genes of pathogenic bacteria [3, 4] and synthesising new genomes [5, 6]. Researchers have used different methods for studying the essentility of genes in prokaryotes. Baba et al. [7] have made a library of single gene deletions using phage lambda Red recombination system to screen essential genes while another group have used antisense RNA knockdowns for this purpose [8]. Another method that is widely used due to its simplicity and accuracy is transposon mutagenesis along with high-throughput sequencing [9, 10, 11, 12, 13, 14, 15]. In this method, pools of single insertion mutants are constructed using transposon mutagenesis and the effect of each mutation on the survival of mutants is evaluated by sequencing the survivors [16]. This can lead to the identification of essential genes.

Although the essentiality of genes has been studied in a variety of organisms, there is still room to study the evolutionary conservation of essentiality. Barquist et al. [17] have used transposon-directed insertion-site sequencing to study the differentiation of the essentiality of genes in *Salmonella* serovars Typhi and Typhimurium which has led to divergence in their pathogenecity and host ranges. We extend this research by studying 13 bacterial strains from Enterobacteriaceae. These strains are depicted in Fig. 1.

Enterobacteriaceae is a family that includes bacteria with different host ranges and pathogenecity found in soil, water, plants, animals and humans [18]. In humans, various strains from this family can cause diarrhoea, septicaemia, urinary tract infection, meningitis, respiratory disease, and wound and burn infection [18]. Besides, they can infect poultry and livestocks and cause financial losses for farmers [18]. Here, we perform a transposon-directed insertion-site sequencing experiment to study the conservation of essentiality of genes in strains from 5 different species in this family.

{A summary of what we have done}

Are transposons biased towards certain positions?

- There is a bias towards the position of the gene (Fig. 2).
- There is a negligible bias towards certain motifs (Fig. 3)
- The G-C bias needs to be studied further (Fig. 4)
- The number of insertions on the 3 and 5 ends is more than the internal region in essential genes and less than the internal region in beneficial losses. (Fig. 5)

Gene classes

Genus specific.

Single copy.

Multi-copy.

Evolution of essentiality

- UpSetR results (Fig. 6)
- Stringent
- Dollo law
- Ancestral insertion index

Case study of genes

Core genes.

Accessory genes.

Discussion

Materials and Methods

Clustering orthologous and paralogous genes. To study the essentiality of genes in 12 our strains, We needed to cluster sets of orthologous genes in these strain. Plenty of methods are proposed for this purpose. Altenhoff et al. have compared 15 of these methods [19] and shown that Hieranoid [20] is among three methods that keep a balance between precision and recall. We have used Hieranoid to cluster the sets of orthologous genes. In addition, we intended to study the essentiality of genes in paralogous genes. For this, we have developed a program that clusters all homologous genes using Jackhmmer from HMMER3 package [21].

ACKNOWLEDGMENTS.

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Reserved for Publication Footnotes

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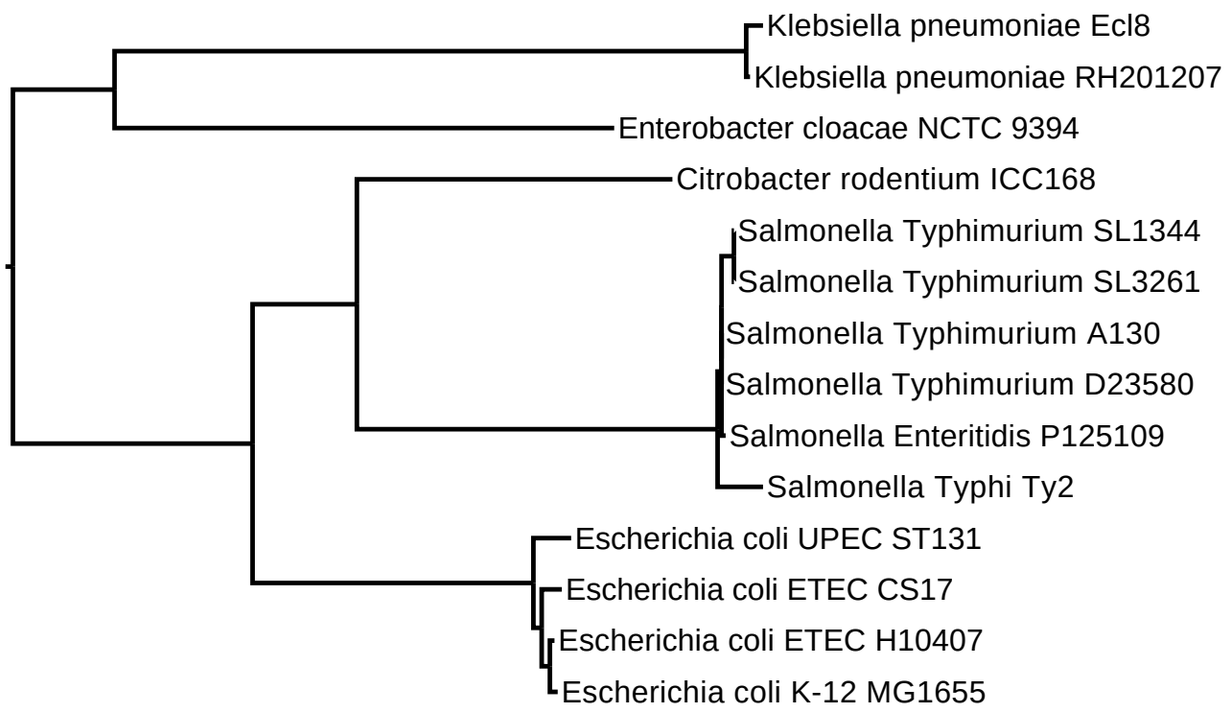

Fig. 1: The species tree containing the 13 strains under study and Escherichia *coli* K-12 MG1655 studied in Keio collection [7]. We have generated the tree by running RAxML [22] on Phylosift [23] amino acid markers.

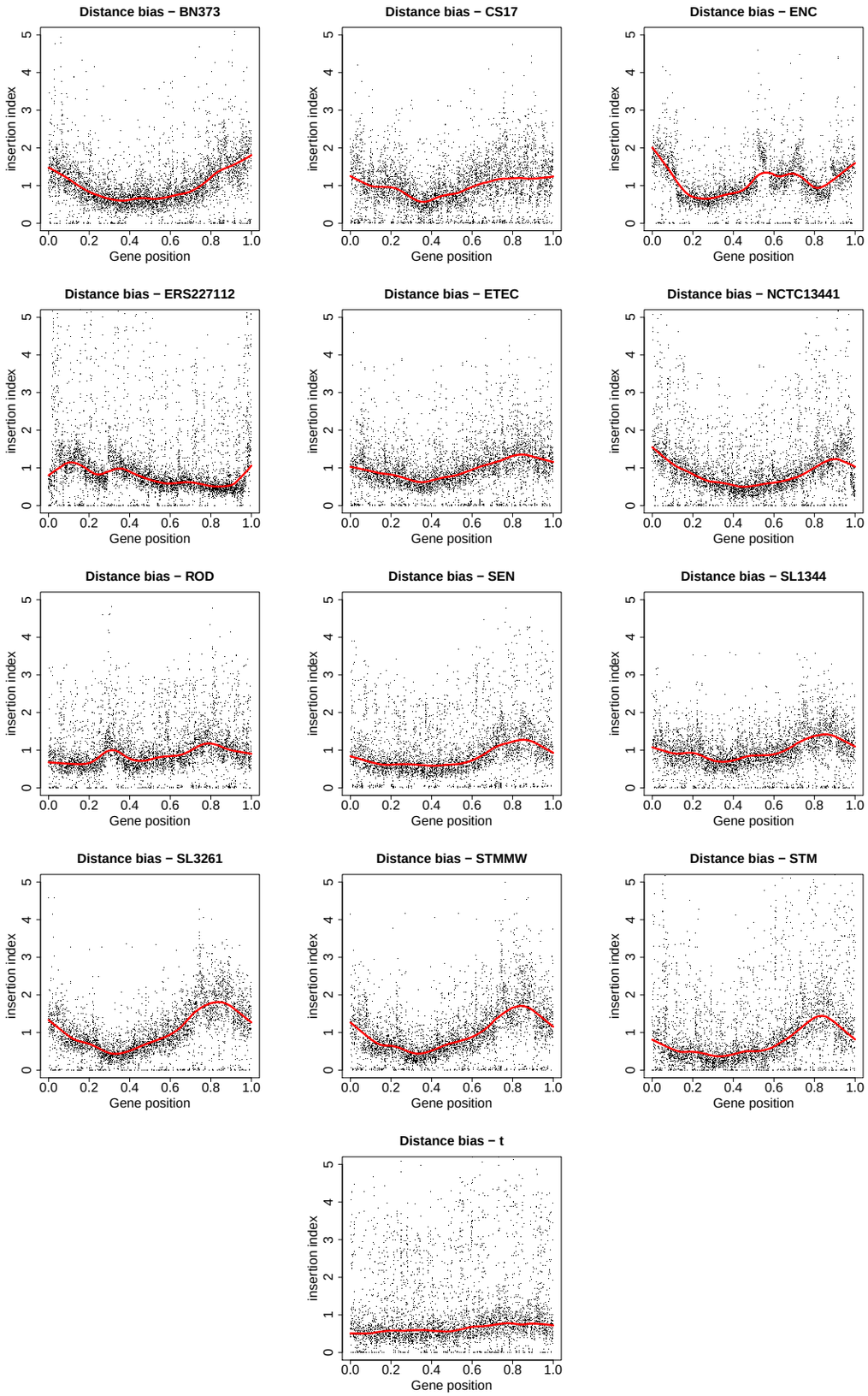

Fig. 2: The plots show the position of the genes within the genome (normalised by the lengths of the genomes) versus the insertion indices of the genes

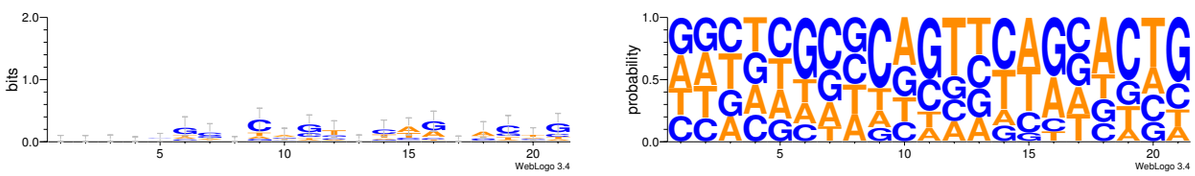

Fig. 3: We have generated the logos from 10 nucleotides flanking the 100 top most frequent insertion sites.

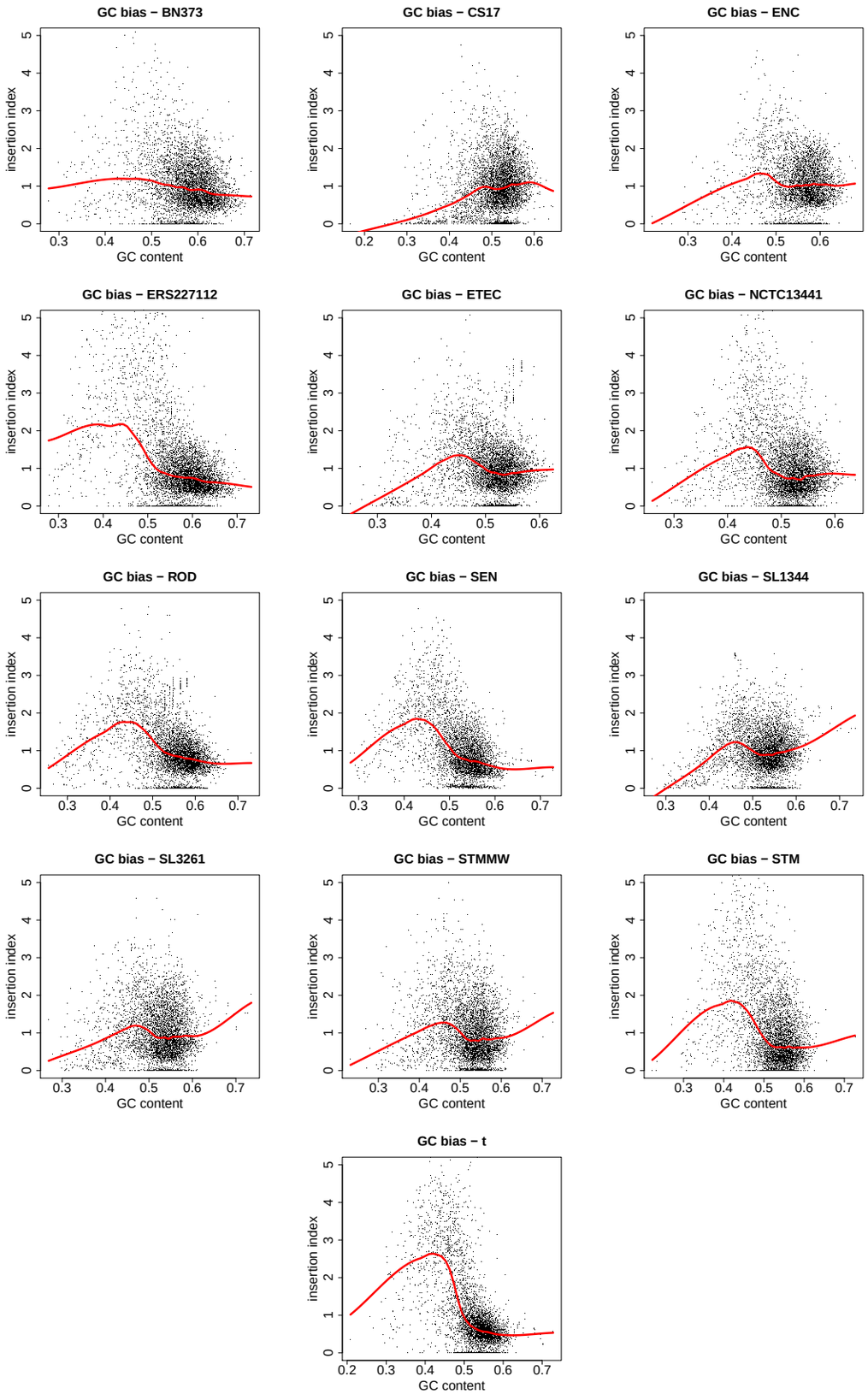

Fig. 4: The plots show the G-C contents of the genes (normalised by the lengths of the genes) against their insertion indices

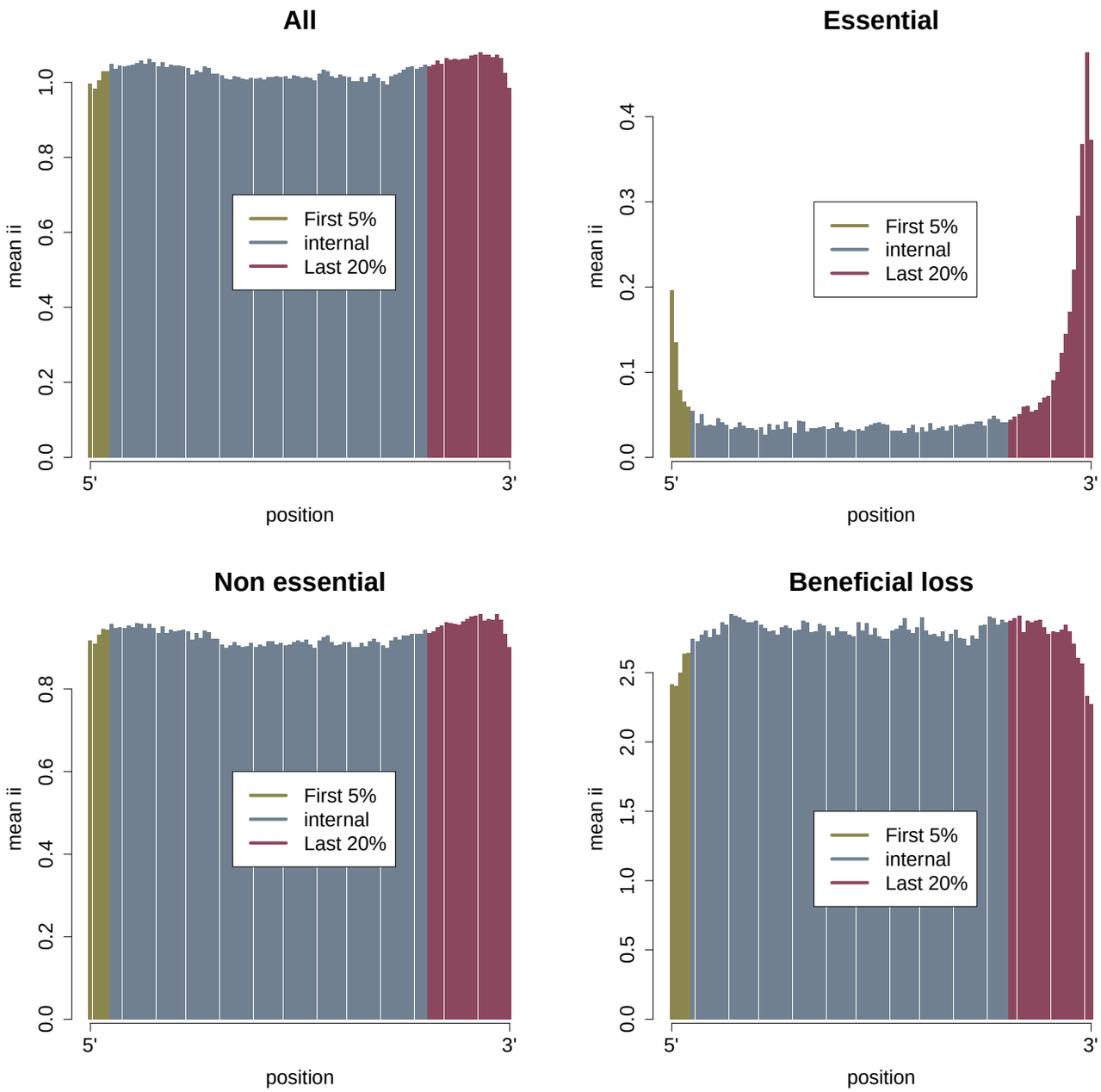

Fig. 5: We have divided our genes into 3 segments: 5% of the genes on the 5 end, 20% of the genes on the 3 end, and the rest in the middle.

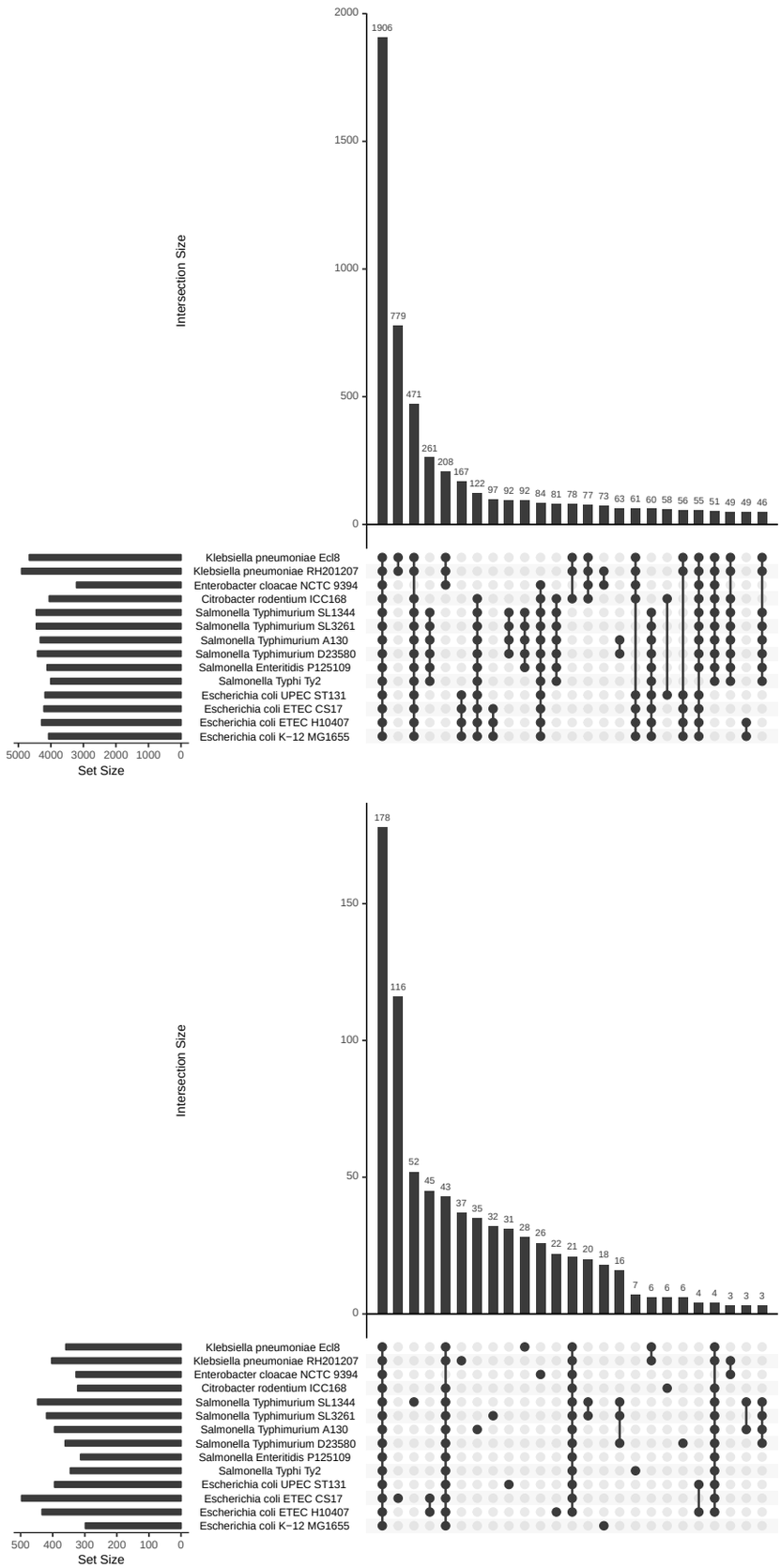

Fig. 6: The first figure shows the number of core genes between each group of species and the second figure shows the number of core essential genes.