

Supplementary Materials

Epidemiological and phylogenetic analysis reveals Flavobacteriaceae as potential ancestral source of tigecycline resistance gene *tet(X)*

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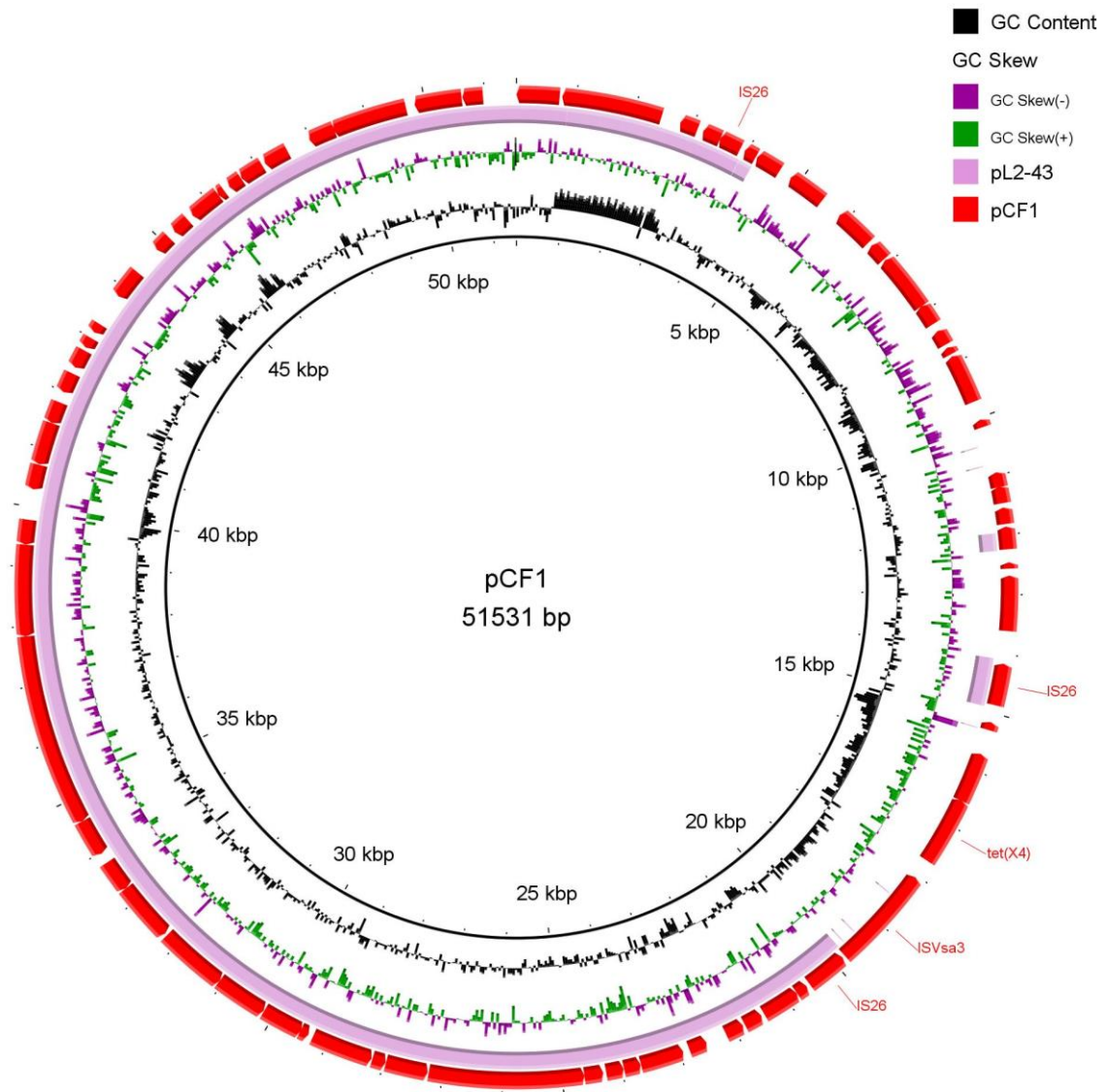
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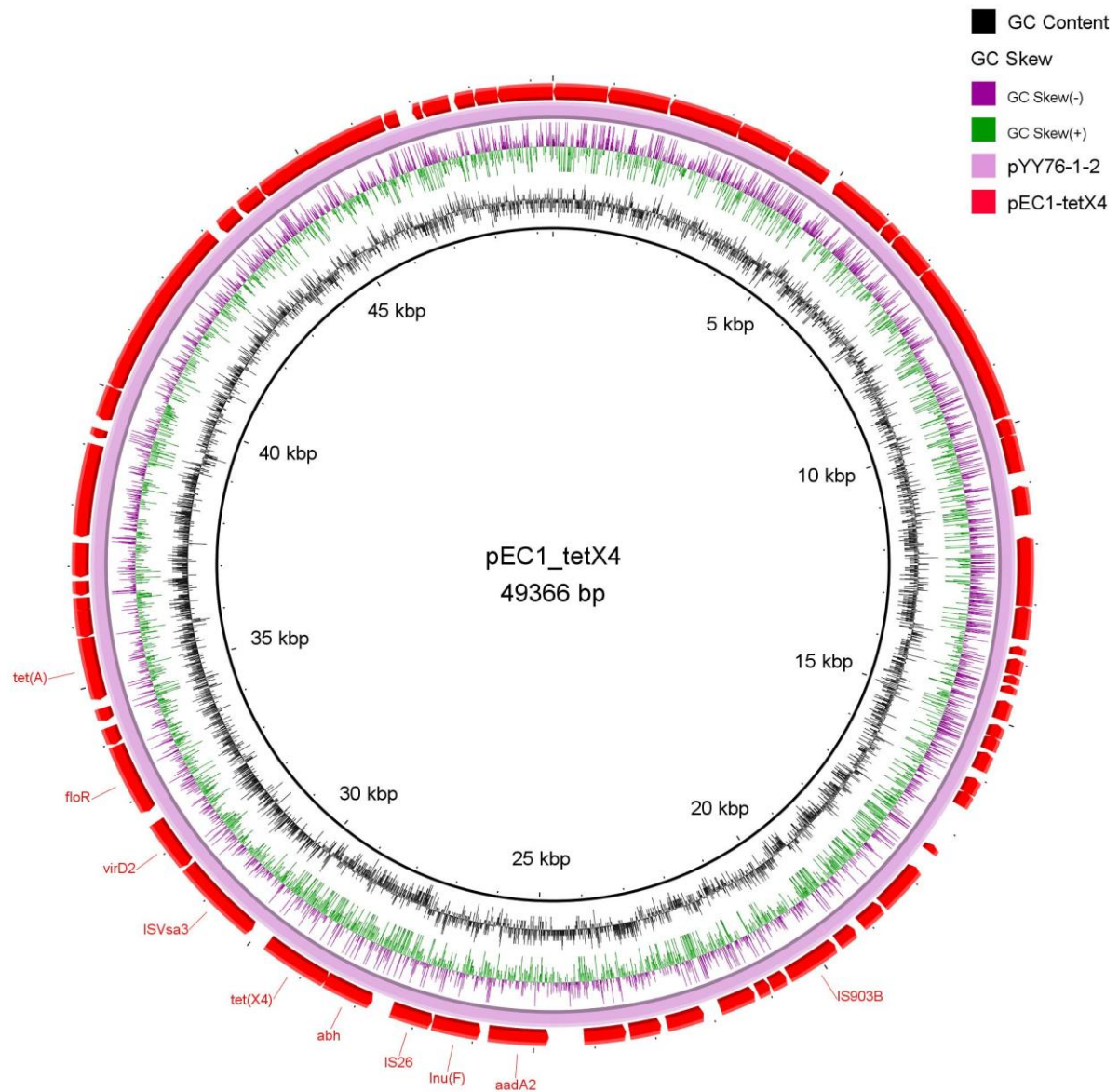
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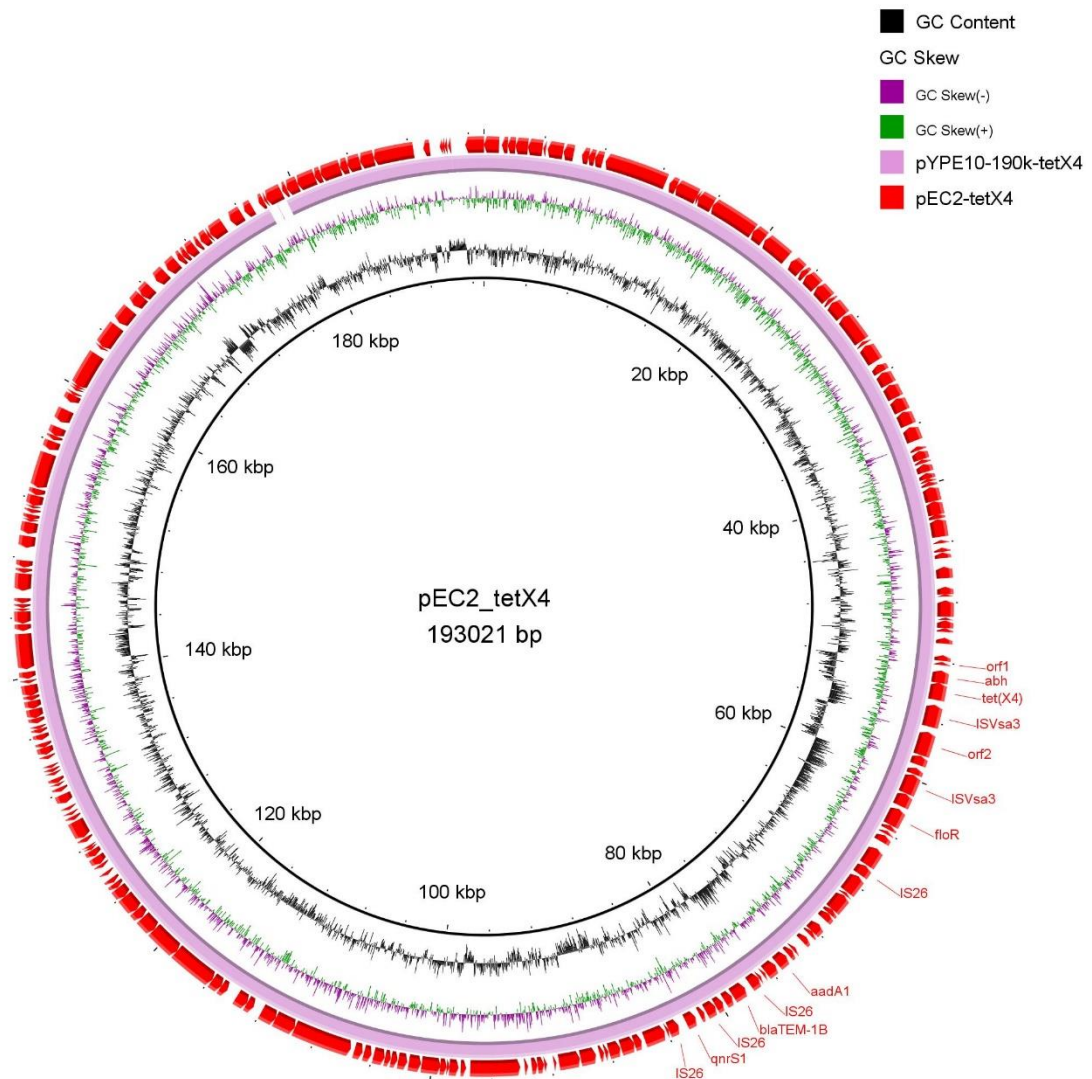
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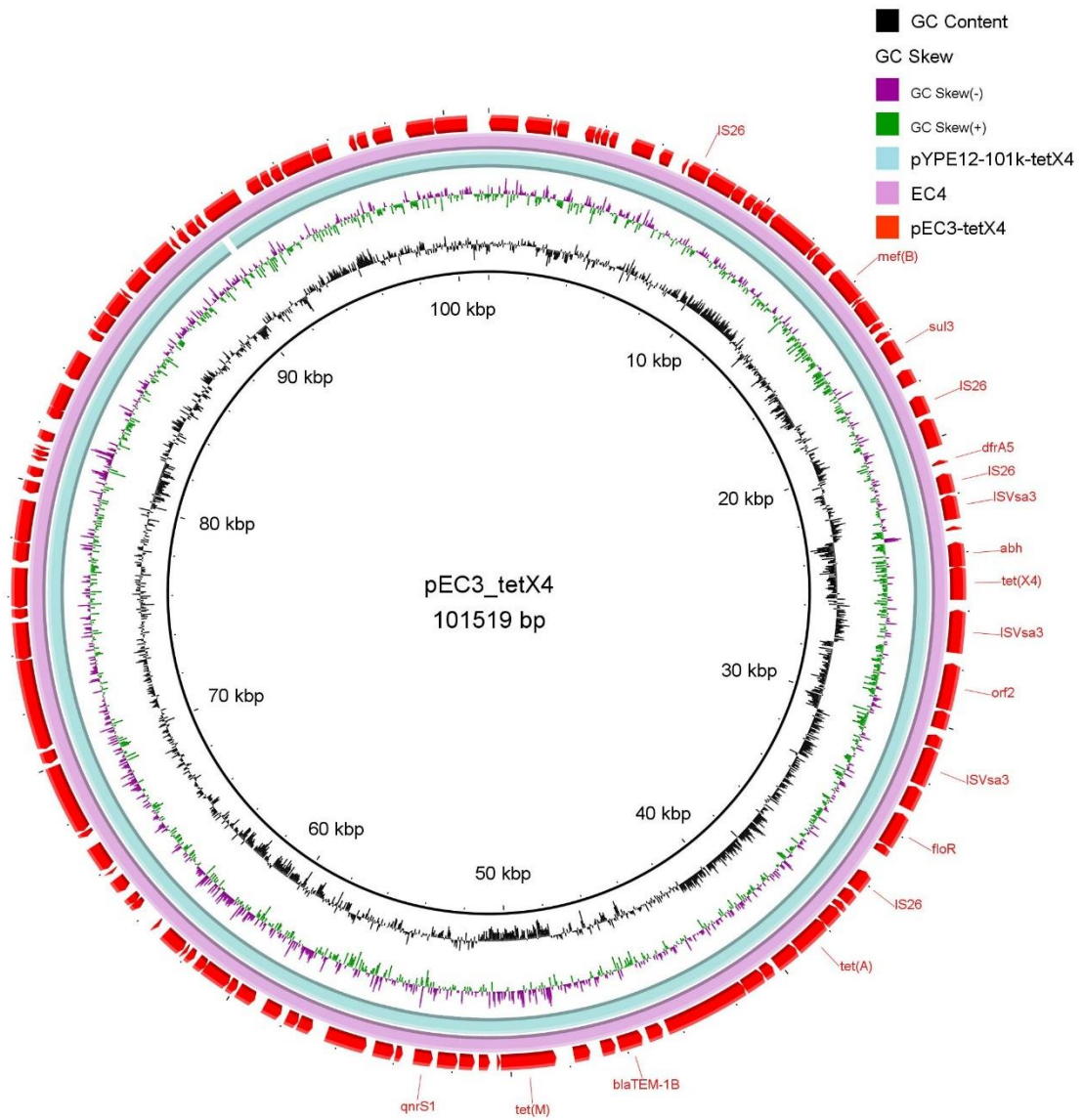
Supplementary Figure 1. Circular map of plasmid pCF1. The inner circle is plasmid pL2-43 (Accession: KJ484641) isolated from an *E. coli* isolate. Insertion sequences and antimicrobial resistance genes are labelled with red fonts.



Supplementary Figure 2. Circular map of plasmid pEC1_tetX4. The inner circle is plasmid pYY76-1-2 (accession: CP040929) isolated from an *E. coli* isolate. Insertion sequences and antimicrobial resistance genes are labelled with red fonts.



Supplementary Figure 3. Circular map of plasmid pEC2_tetX4. The inner circle is plasmid pYPE10-190k-tetX4 (accession: CP041449) isolated from an *E. coli* isolate. Insertion sequences and antimicrobial resistance genes are labelled with red fonts.



Supplementary Figure 4. Circular map of plasmid pEC3_tetX4. The inner circle is plasmid pYPE12-101k-tetX4 (accession: CP041443) isolated from an *E. coli* isolate. Insertion sequences and antimicrobial resistance genes are labelled with red fonts.

Supplementary Table 1. Primers used in this study

gene	Primer name	Primer sequence	Product size	purpose	Reference
<i>tet(X)</i>	tetXF	TTAGCCTTACCAATGGGTGT	243 bp	<i>tet(X)</i> -positive bacteria screening	¹
	tetXR	CAAATCTGCTGTTTCACTCG			
<i>tet(X3)</i>	tetX3F	CAGGACAGAAACAGCGTTGC	179 bp	gene expression level analysis	²
	tetX3R	GCAGCATCGCCAATCATTGT			
<i>tet(X5)</i> -variant	tetX5VF	TGCCTTGATATTGTCGGATAA	113 bp	gene expression level analysis	this study
	tetX5VR	ATTCTGCCTGTGCTTCTC			

References

- 1 Bartha, N. A., Sóki, J., Urbán, E. & Nagy, E. Investigation of the prevalence of tetQ, tetX and tetX1 genes in Bacteroides strains with elevated tigecycline minimum inhibitory concentrations. *International journal of antimicrobial agents* **38**, 522-525 (2011).
- 2 Li, Y., Shen, Z., Ding, S. & Wang, S. A TaqMan-based multiplex real-time PCR assay for the rapid detection of tigecycline resistance genes from bacteria, faeces and environmental samples. *BMC microbiology* **20**, 1-7 (2020).