Supplementary Information

Horizontal gene transfer and acquired antibiotic resistance in *S.* Heidelberg following *in vitro* incubation in broiler ceca

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Text S1

Benchmarking of resistome enrichment

Resistome dataset was benchmarked against WGS data of susceptible and MDR strains of *S.* Heidelberg (n= 2), *S.* Kentucky (n= 3), *S.* Enteritidis (n= 1), *Campylobacter jejuni* (n= 3), *Campylobacter coli* (n= 4) and *E. faecalis* (n= 4). These strain were collected as part of an on-going AMR monitoring project. Antimicrobial susceptibility testing, WGS and resistome enrichment was done for each isolate. De novo assembly was done on WGS and resistome Fastq reads using SPaDes. Contigs were queried against ResFinder for acquired ARG determination and against CARD for global transcriptional regulator histone-like nucleoid structuring (H-NS).

We chose this reference gene because it is present in the majority of Gram-negative bacteria including *E. coli* at high copies per genome and plays a central role in the silencing of newly acquired genes or mobile elements (1). Consequently, H-NS is required for the regulation of ~ 5% of *E. coli* genes including plasmid or phage genes (1, 2). Therefore, ARG's that are inactive or silenced will be lower in abundance compared to H-NS. The relative abundance of an ARG (log2 fold-change) was determined from the coverage of the contig carrying the ARG, normalized against the coverage of H-NS. This calculation may be biased towards Gram-positive bacteria that are not known to harbor H-NS. Lastly, we performed a correlation test (Kendall Tau) to evaluate the relationship between WGS-derived and enrichment-derived relative abundance.

Illumina short read and MinION long read hybrid assembly

Sequencing was performed with both MiSeq (Illumina, Inc. San Diego, CA) and MinION (Oxford Nanopore Technologies Ltd, Oxford, UK) to obtain short and long reads respectively in fastq

format. Initial read number and read quality for the illumina reads was assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) on default settings and compiled into a report using MultiQC (https://github.com/ewels/MultiQC). Porechop (https://github.com/rrwick/Porechop) was used to demultiplex the MinION reads into their respective samples via barcode and read quality was assessed with NanoPlot (http://nanoplot.bioinf.be/). Trimmomatic (3) was used for read trimming on the illumina short-read data with a phred quality score of 15. Both trimmed and non-trimmed data, along with MinION long-reads, were then assembled with SPAdes using the '--careful' option, resulting in a hybrid assembly. QUAST, followed by MultiQC, was used to generate a report of assembly statistics using reference genomes relevant to each sample.

Table S1. Primers used for this study.

Assay name	Primer	Primer sequence (5 '-3')	Target Gene	Genome	Amplification factor	Melt curve (T_m)	Reference
gapA	F	TTGGAGATGTGAGCAATC	Glyceraldehyde-6-dehydrogenase	Chromosome	1.93	80.5 °C	(4)
	R	GACAACTTCGTGAAACTG					
repB_IncX1	F	TGGACATACGAAGAAGAG	Pir Family of Replicase	Plasmid	1.90	79.5 °C	(4)
	R	AACCTGAGTAGTGTAAGAAT					
IncK2	F	CTTATCTTATCTATTGCCACATA	*inc*RNAi - replication protein	Plasmid	1.99	83 °C	This study
	R	CACTCTTGTTAGCCTTGT					
IncK2_marta	F	ATGCTCGCGGTCCGGAAAGCC	*inc*RNAi - replication protein	Chromosome	ND	81.0 °C	(5)
	R	GTGCCGTGCGTTAATGCACTGCAA					
repL	F	CCAATCAACCGTCGTTCGTG	Lytic replication gene	Chromosome	2.63	88.5 °C	(6)
	R	TAAGCATATTTCCGCGCTGC					
bla_{CMY-2}	F	ACTCCGGGCGCTAAGCGACTTTAC	CMY-2	Plasmid	1.85	87.5 °C	(7)
	R	CGCCAATACGCCAGTAGCGAGACT					

Note.

F- Forward primer, R - Reverse primer , ND- Not determined

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81 **Table S2:** Mutations acquired by *S.* Heidelberg isolates following incubation in broiler ceca [a].

Loci on reference genome	Location [b]	Product	Mutation change [c]
551931	**Intragenic**	**Endopeptidase La**	**ND**
598142	Intergenic	Before Kef family K (+) transporter, after fosmidomycin resistant protein	N/A
933383	Intragenic	ATP-dependent DNA helicase (DinG)	ND
933947	**Intragenic**	**ATP-dependent DNA helicase (DinG)**	**ND**
1520727	Intragenic	Transcriptional regulator (SlyA)	Asp84Gly
1544026	Intragenic	Fumarate hydratase	Asp416Ala
2349475	Intragenic	Thiol:disulfide interchange protein	Val100Gly
2688401	**Intragenic**	**Fe-S protein assembly chaperone (HscA)**	**ND**
2828163	**Intragenic**	**Phage integrase (Int)**	**ND**
2889256	Intragenic	MprA	Trp140Arg
3072149	Intragenic	Cysteine sulfinate desulfinase	Val116Val
3503197	ITS	23S - 5S rRNA ITS	N/A
3503199	ITS	23S - 5S rRNA ITS	N/A
3503200	ITS	23S - 5S rRNA ITS	N/A
3503201	ITS	23S - 5S rRNA ITS	N/A
3503202	ITS	23S - 5S rRNA ITS	N/A
3503203	ITS	23S - 5S rRNA ITS	N/A
3535133	Intragenic	Translation elongation factor Tu	ND
3594515	Intragenic	Two-component system sensor histidine kinase (EnvZ)	ND

3948989	**Intragenic**	**C-type cytochrome biogenesis protein (CcmF)**	**Met550Val**
3956264	Intragenic	Trimethylamine N-oxide reductase I catalytic subunit (TorA)	ND
4032168	**ITS**	**16S - 23S rRNA ITS**	**N/A**
4087617	Intragenic	Magnesium and cobalt transport protein (CorA)	ND
4087651	Intragenic	Magnesium and cobalt transport protein (CorA)	ND
4193409	**Intragenic**	**Two-component system sensor histidine kinase (CpxA)**	**ND**
4271745	Intragenic	rRNA-23S ribosomal RNA	N/A
4271746	Intragenic	rRNA-23S ribosomal RNA	N/A
4271748	Intragenic	rRNA-23S ribosomal RNA	N/A
4271751	Intragenic	rRNA-23S ribosomal RNA	N/A

Notes.

[a] Mutations present in SH-2813$_{nal}$ isolates recovered from broiler ceca but absent in parental isolates are reported. SNPs/indels were compared to reference genome CP016573. Boldness denotes mutations unique to isolates that acquired multidrug resistance.

[b] Intragenic - present within a coding sequence, Intergenic - Non-coding region between two genes, ITS - internal transcribed spacer.

[c] N/A - Not available (SNP/indel present in non-coding sequence), ND - not determined (indel present).

Table S3: Antibiotic resistance genes and efflux pumps encoded on the chromosome of SH-2813$_{nal}$.

Gene	Drug Class	Resistance Mechanism
AAC(6')-Iaa	Aminoglycosides	antibiotic inactivation
acrB	Multidrug	antibiotic efflux
adeF	Tetracyclines, fluoroquinolones	antibiotic efflux
bacA	Peptides	antibiotic target alteration
baeR	Aminoglycosides, aminocoumarins	antibiotic efflux
cpxA	Aminoglycosides, aminocoumarins	antibiotic efflux
CRP	Multidrug	antibiotic efflux
emrB	Fluoroquinolones	antibiotic efflux
emrR	Fluoroquinolones	antibiotic efflux
acrA	Multidrug	antibiotic efflux
EF-Tu	Elfamycin	antibiotic target alteration
GlpT	Fosfomycin	antibiotic target alteration
marR	Multidrug	antibiotic efflux; antibiotic target alteration
mdfA	Tetracyclines, benzalkonium chloride, rhodamine	antibiotic efflux
nfsA	Nitrofurans	antibiotic target alteration
soxR	Multidrug	antibiotic efflux; antibiotic target alteration
soxS	Multidrug	antibiotic efflux; reduced permeability to antibiotic; antibiotic target alteration
UhpT	Fosfomycin	antibiotic target alteration
FosA7	Fosfomycin	antibiotic inactivation
golS	Multidrug	antibiotic efflux
H-NS	Multidrug	antibiotic efflux
PBP3	Beta-lactamase	antibiotic target alteration
kdpE	Aminoglycosides	antibiotic efflux

marA	Multidrug	antibiotic efflux; reduced permeability to antibiotic
mdsA	Beta-lactamase and phenicols	antibiotic efflux
mdsC	Beta-lactamase and phenicols	antibiotic efflux
MdtK	Fluoroquinolones	antibiotic efflux
msbA	Nitroimidazoles	antibiotic efflux
patA	Fluoroquinolones	antibiotic efflux
PmrF	Peptides	antibiotic target alteration
sdiA	Multidrug	antibiotic efflux

Notes.

The Comprehensive Antibiotic Resistance Database (CARD) was used for the identification of resistance and efflux pumps present on chromosome.

Table S4: Antibiotic resistance genes detected with WGS and resistome enrichment.

Bacteria isolate	ARG's detected by WGS	ARG's detected by targeted enrichment of ARG's	Resistance phenotype [c]
Campylobacter coli_7	*blaOXA-184, tet(O)*	*blaOXA-184, tet(O)*	AZI, CIP, CLI, ERY, NAL, TEL, TET
Campylobacter coli_10	*aph(3')-III, aph(2'')-Ig, tet(O)*	*aph(3')-III, aph(2'')-Ig, tet(O)*	AZI, CLI, ERY, GEN, TEL, TET
Campylobacter coli_12	*aph(3')-III, blaOXA-450, tet(O)*	*aph(3')-III, blaOXA-450, tet(O)*	AZI, CIP, CLI, ERY, NAL, TEL, TET
Campylobacter coli_13	*aph(2'')-Ig, aph(3')-III, tet(O)*	*aph(2'')-Ig, aph(3')-III, tet(O)*	AZI, CLI, ERY, GEN, TET
Campylobacter jejuni_8	*blaOXA-450, tet(O)*	*blaOXA-450, tet(O)*	AZI, CIP, ERY, GEN, NAL, TET
Campylobacter jejuni_9	*blaOXA-450, tet(O)*	*blaOXA-450, tet(O)*	AZI, CIP, CLI, ERY, NAL, TEL, TET
Campylobacter jejuni_11	*blaOXA-450, tet(O)*	*blaOXA-450, tet(O)*	AZI, CIP, CLI, ERY, NAL, TEL, TET
Enterococcus faecalis_15	*aac(6')-aph(2''), lsa(A)[a], tet(L), tet(M)*	*aac(6')-aph(2''), lsa(A), tet(L), tet(M)*	GEN, KAN, LIN, TET
Enterococcus faecalis_16	*lsa(A)[a], tet(O)*	*lsa(A), tet(O)*	LIN, TET
Enterococcus faecalis_17	*lsa(A)[a], tet(M), tet(L)*	*lsa(A), tet(M), tet(L)*	LIN, TET

Enterococcus faecalis_14	*lsa(A)*[a]	*lsa(A)*	LIN
Salmonella Enteritidis_6	*aac(6')-Iaa*[b], *aac(3)-Via, aadA1, blaTEM-1B, sul1*	*aac(6')-Iaa*[b], *aac(3)-Via, aadA1, blaTEM-1B, sul1*	AMP, GEN, STR, SMX
Salmonella Heidelberg_5	*aac(6')-Iaa*[b], *ant(2'')-Ia, aph(3'')-Ib, aph(6)-Id, blaCMY-2, cmlA1, sul2*	*aac(6')-Iaa, ant(2'')-Ia, aph(3'')-Ib, aph(6)-Id, blaCMY-2, cmlA1, sul2*	AMC, AMP, FOX, TIO, CRO, CHL, GEN, KAN, STR, SMX
Salmonella Kentucky_1	*aac(6')-Iaa*[b], *aph(3'')-Ib, aph(6)-Id, blaCMY-2, tet(B)*	*aac(6')-Iaa, aph(3'')-Ib, aph(6)-Id, blaCMY-2, tet(B)*	AMC, AMP, FOX, TIO, CRO, STR, TET
Salmonella Kentucky_2	*aac(6')-Iaa*[b], *aph(3'')-Ib, aph(6)-Id, blaCMY-2, tet(B)*	*aac(6')-Iaa, aph(3'')-Ib, aph(6)-Id, blaCMY-2, tet(B)*	AMC, AMP, FOX, TIO, CRO, STR, TET
Salmonella Kentucky_3	*aac(6')-Iaa*[b], *aph(6)-Id, aph(3'')-Ib, sul2, dfrA14*	*aac(6')-Iaa, aph(6)-Id, aph(3'')-Ib, sul2, dfrA14*	SMX, COT
Salmonella Kentucky_4	*aac(6')-Iaa*[b], *aph(3'')-Ib, aph(6)-Id, tet(B)*	*aac(6')-Iaa, aph(3'')-Ib, aph(6)-Id, tet(B)*	AMC, AMP, FOX, TIO, CRO, CHL, KAN, STR, SMX, TET

[a] *lsa(A)* gene was identified on the chromosome of *E. faecalis* strains.

[b] *aac(6')-Iaa* gene was identified on the chromosome of *Salmonella* strains.

[c] Abbreviations: Amc - Amoxicillin/Clavulanic acid, Amp – Ampicillin, Azi - Azithromycin, Chl – Chloramphenicol, Cip - Ciprofloxacin, Cli – Clindamycin, Cot - Trimethoprim-Sulfamethoxazole, Cro – Ceftriaxone, Ery – Erythromycin, Fox - Cefoxitin, Gen – Gentamicin, Kan - Kanamycin, Lin – Lincomycin, Nal – Nalidixic acid, Smx-Sulfamethoxazole, Str - Streptomycin, Tel – telithromycin, Tet – Tetracycline, Tio - Ceftiofur,

Table S5: Illumina/MinION hybrid assembly statistics and plasmids identified.

Strain ID (phylogroup) [a]	N50 (bp)		Largest contig (bp)		Plasmids (ARG)	Plasmid contig size (bp) [c]
	Illumina short read assembly	Illumina/MinION hybrid assembly	Illumina short read assembly	Illumina/MinION hybrid assembly [b]		
Ec15ceca (A)	59,000	4,728,800	35,500	4,728,800	IncFIB (tetA)	100,085
					Col440i	3,384
Ec6BL (D)	145,000	462,700	291,000	1,050,400	IncK2 (CMY-2)	88,889 (C)
					IncF4:A5:B1 (TEM-1B)	168,225 (C)
					IncFII (aadA1, aac(3)-Via, tetA, sul1)	117,389
					Col (MG828)	1,700
Ec15BL (E)	100,000	608,100	268,100	1,373,000	IncK2 (CMY-2)	85,509
					IncF4:A-:B1	144,103
					Col (MG828)	2,439

Notes:

[a]Phylogroup was determined using ClermonTyping (http://clermontyping.iame-research.center/).

[b]Hybrid assembly was done using Illumina short reads and MinION long reads. Hybrid assembly was used for antibiotic resistance gene (ARG) and plasmid identification. ARG carried by each plasmid are in parentheses.

[c]Plasmid contigs that are complete and circular (C) are identified.

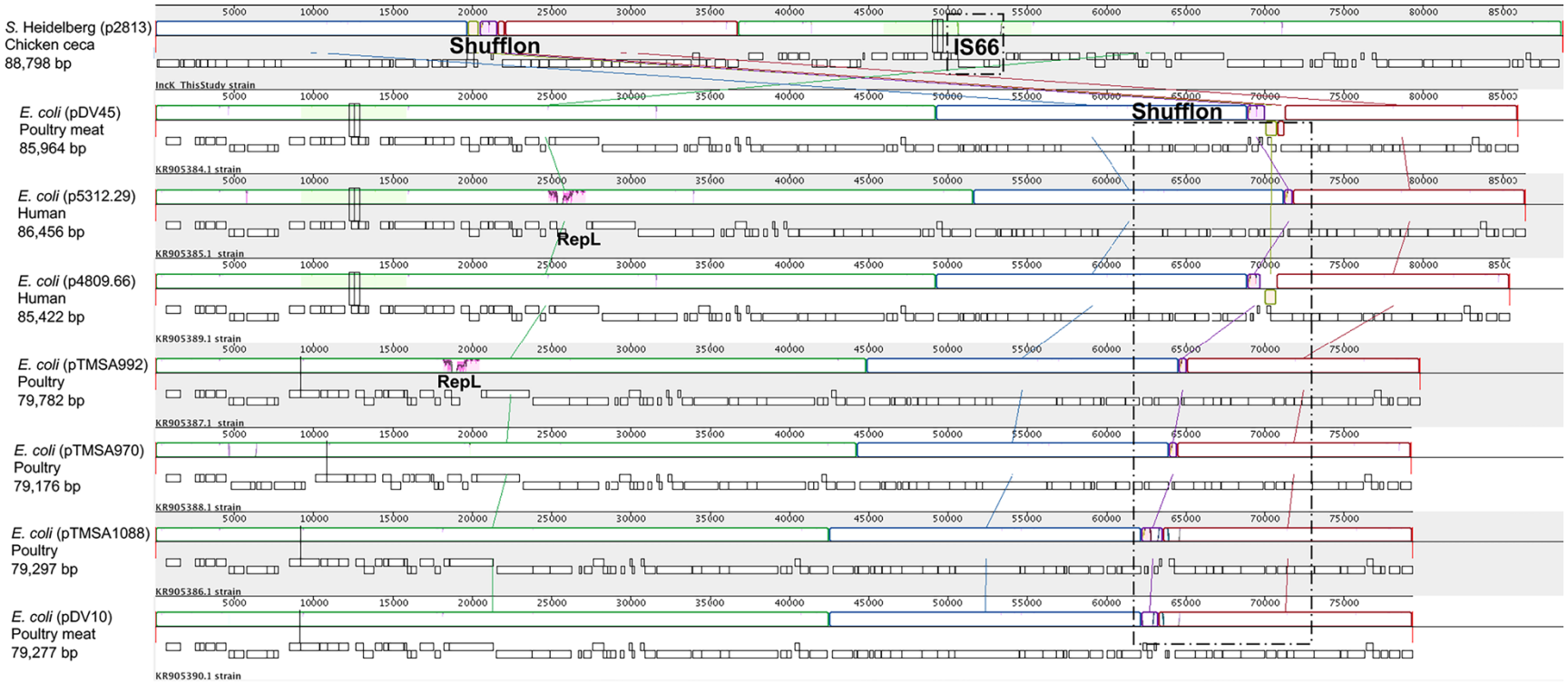

Fig. S1 ProgressiveMauve alignment of IncK2 complete plasmid DNA sequences from this study and Seiffert et al (8). DNA regions that differ between the Inck2 from this study and Seiffert et al (8) are highlighted with dashed horizontal black rectangular boxes.

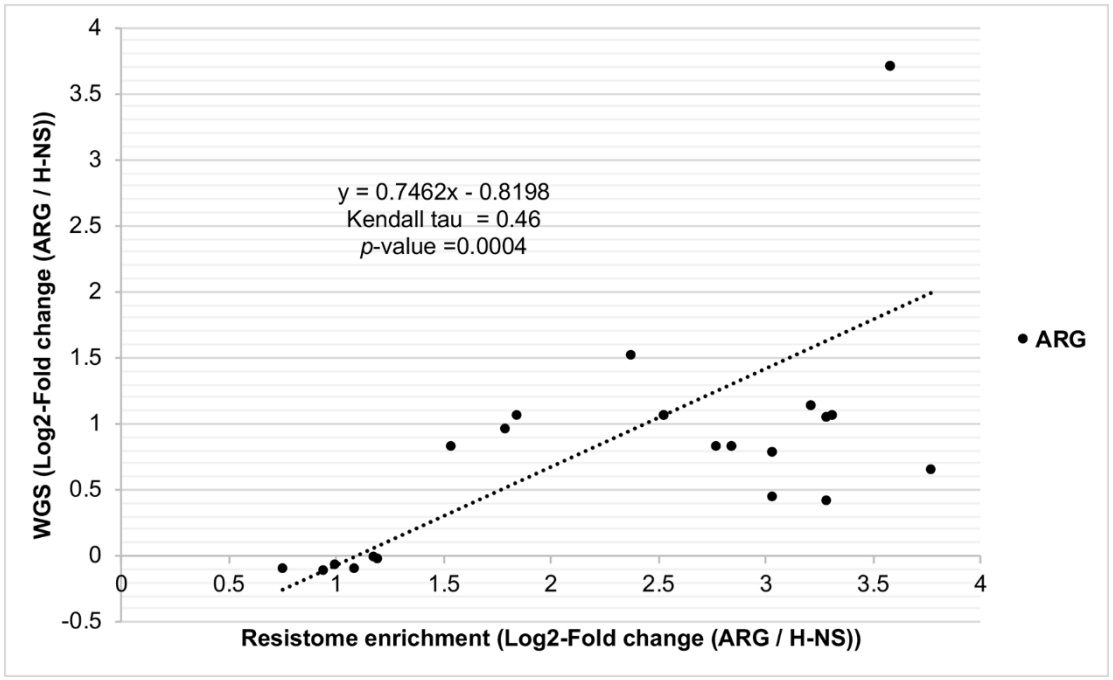

Fig. S2 Correlation between WGS and resistome enrichment-based relative abundance determination. ARG abundance was calculated by dividing the ARG contig coverage by the coverage of H-NS. ARG's (n =31) present in MDR *Salmonella* serovars (n =5) was used for Kendall tau's correlation test.

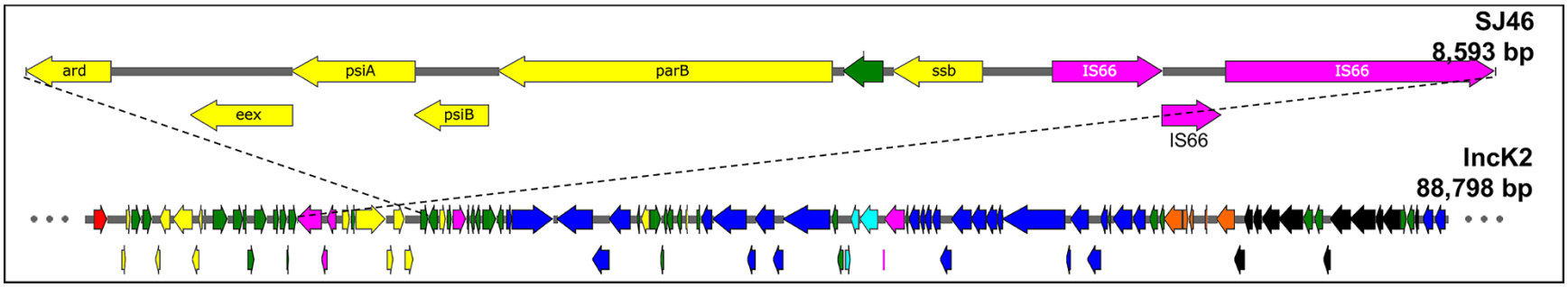

Fig. S3 Annotated map of predicted P1-like phage genome present in the IncK2 plasmid from this study. Dashed diagonal black lines denotes phage SJ46 region identified by PHAST. Map was drawn using SnapGene v.4.3.8.1.

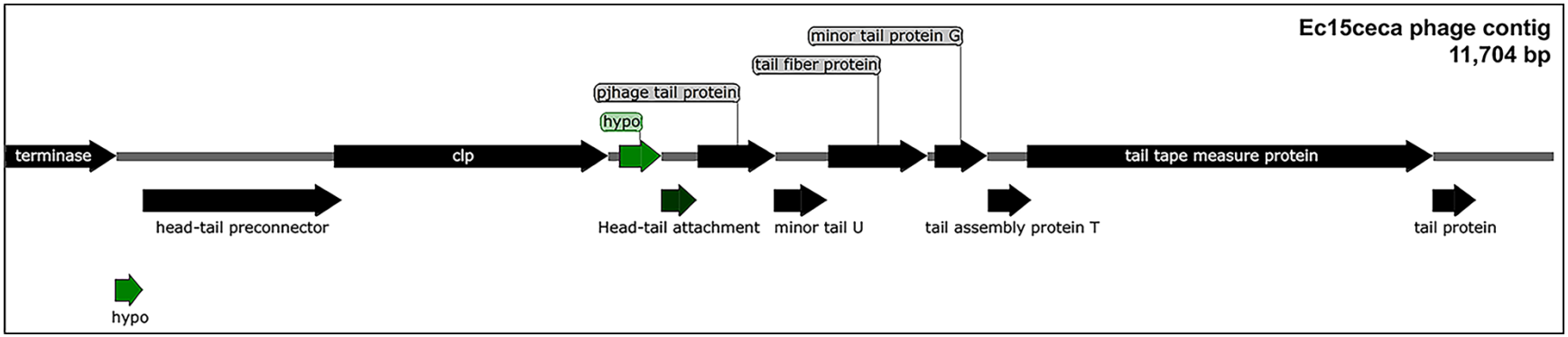

Fig. S4 Annotated mEp460 phage contig of strain Ec15ceca sharing homology with IncK2 *inc*RNAi-rep region. The *inc*RNAi-rep sequence of IncK2 plasmids was queried (tblastx) against the de novo assembled genome of Ec15ceca. Map was drawn using SnapGene v.4.3.8.1.

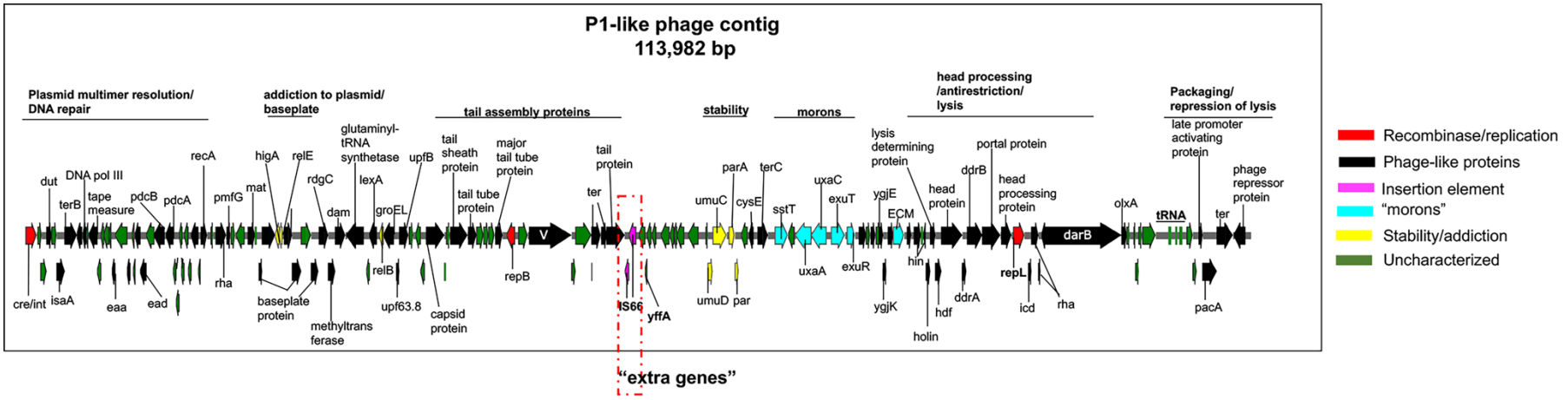

Fig. S5 P1-like phage identified in *E. coli* genome by PHAST**.** Dashed red rectangular box is to highlight "extra genes" discussed in manuscript.

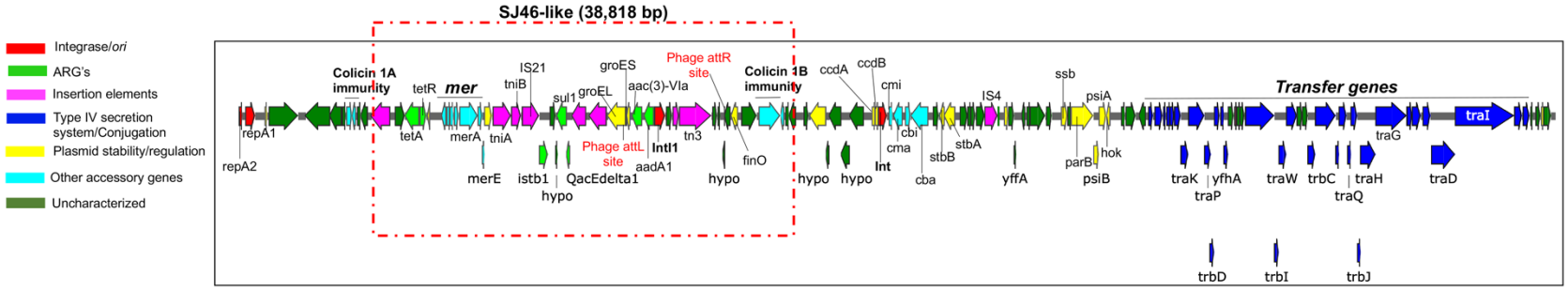

Fig. S6 Annotated map of putative IncFII contig (119,056 bp) transferred *in vitro* from *E. coli* to *S.* Heidelberg. Dashed rectangular red box denotes "predicted" P1-like phage region identified by PHAST. Map was drawn using SnapGene v.4.3.8.1.

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