Supplementary Data for:

"Complete assembly of *Escherichia coli* ST131 genomes using long reads demonstrates antibiotic resistance gene variation within diverse plasmid and chromosomal contexts"

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Supplementary Results

Oxford Nanopore long read quality control and long read genome assembly

Half of the reads were produced within 14 hours of sequencing, with the remainder produced over the subsequent 34 hours (Supplementary Figure 1d). A median read length of 5.5 Kb for reads Q (quality) score $\geq$ 7 was achieved within one hour of sequencing (Supplementary Figure 1e), and the median Q score declined slightly as the run proceeded (Supplementary Figure 1f). An average of 30-fold theoretical coverage from 954 Mbases with Q $\geq$ 7 was exceeded in this GridION run within three hours.

We compared short read-only, long read-only and hybrid assembly outputs from Unicycler v.4.6 using the long Oxford Nanopore reads and short Illumina reads to identify the most contiguous assemblies per sample across all three Unicycler modes (conservative, normal and bold).

For five samples, the long read assemblies produced 2-7 contigs (with a median of three) with nearly identical results across modes, whereas the short read assemblies resulted in 76-230 contigs (a median of 124), and the hybrid assemblies also had more contigs (6-191 with a median of 44). For VREC0739 and VREC1428, the short read libraries resulted in over-bridging of contigs making it harder to classify contigs as chromosomal or plasmid-associated, perhaps because long reads already provided sufficient genome coverage and the assembler inserted the contigs produced by short reads at short homologous repetitive regions.

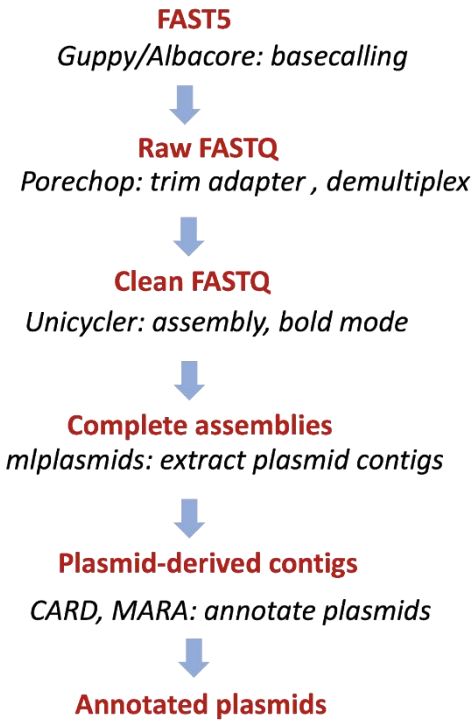
VREC1013 assembly assessment and improvement

For VREC1013, the hybrid assembly improved the long read assembly such that the final optimised version had three rather than 22 contigs and a smaller length (5.36 Mb, Supplementary Table 3), after manual sequence alignment eliminated seven false-positive short contigs. Five contigs had depths of coverage <8% of the chromosomal median and may were the result of contig overbridging during assembly. Pairwise alignment of these five contigs with BLAST against the assembly showed that they had near-perfect matches (E-value < E-10) with other contigs, showing that they were effectively duplicate contigs, and thus few reads mapped to them. In contrast, the other four valid contigs acted positive controls and showed high homology to their own contigs only. As a result, duplicate contigs were removed from the VREC1013 hybrid assembly used for subsequent analyses.

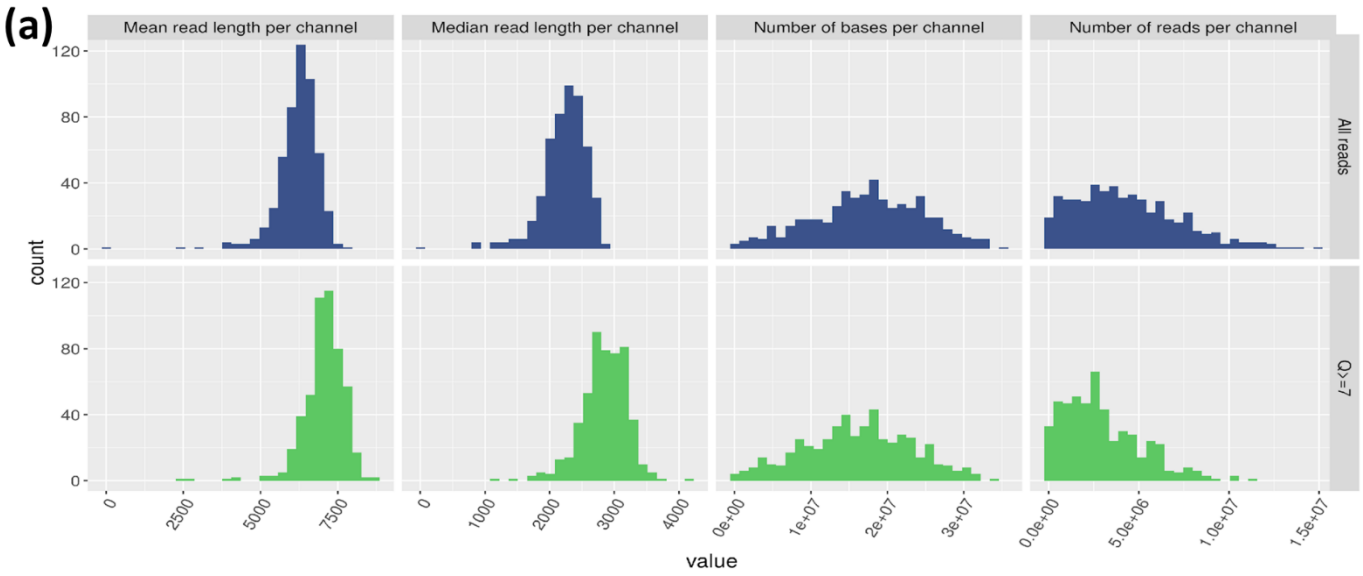
Long plasmid homology search and alignment

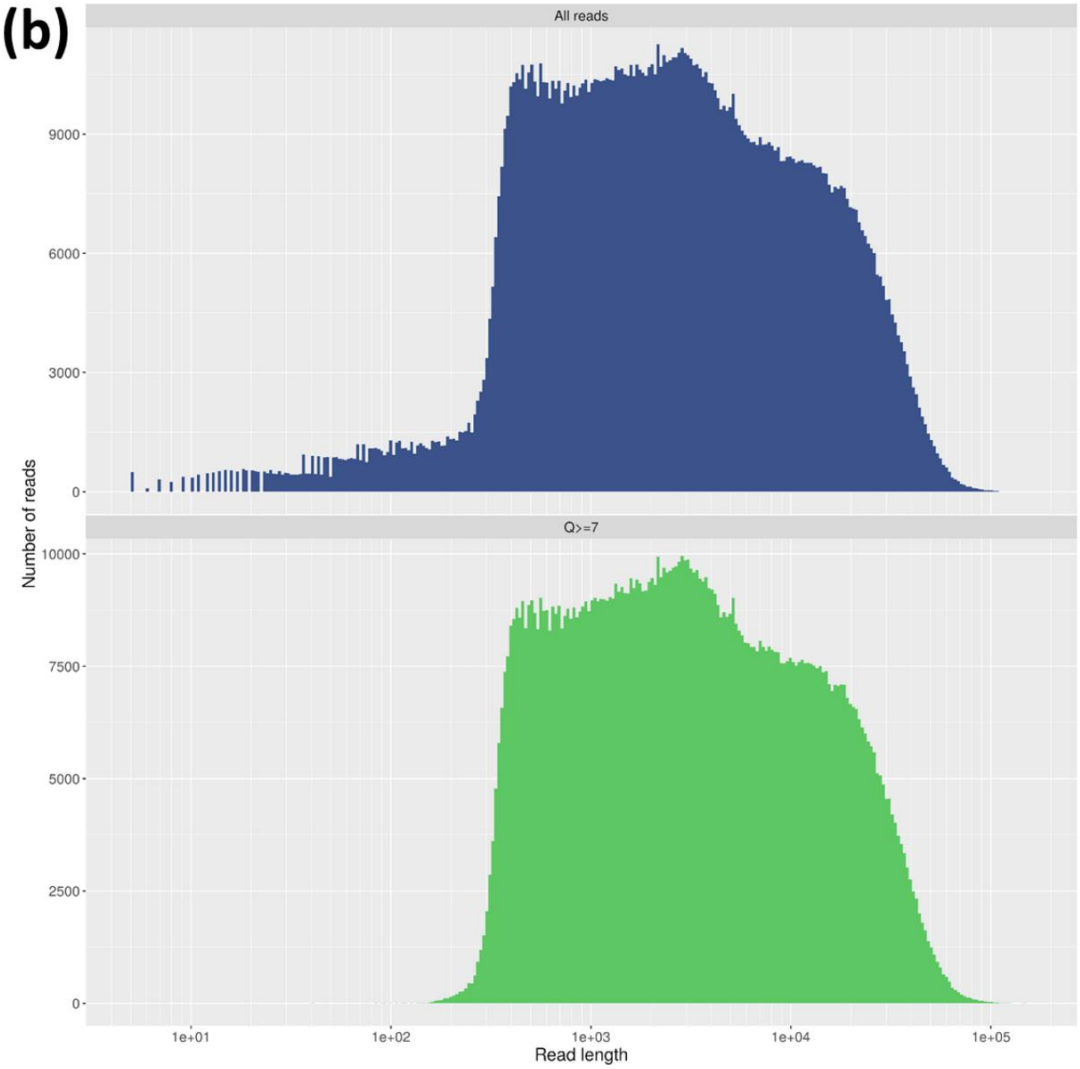
We examined the six long contigs (lengths > 20 Kb) classified as plasmid-derived by aligning them with a database of 10,892 complete plasmids [37] to identify the most similar plasmids using BLAST matches spanning more than one gene (match length > 1,000 bp) with a sequence ID threshold of 95%. This showed the most similar plasmids were isolates were spread across *Enterobacteriaceae* for five and one was in Gammaproteobacteria *Shewanella bicestrii* (VRES1160's plasmid), and that relatively high matching levels were detected for VREC0693's and VREC1428's plasmids, but not for VREC1013, VREC1073 nor VRES1160. The best match to *bla*$_{\text{CTX-M-15}}$-positive VRES1160's IncFIA 61,934 bp plasmid was to *S. bicestrii* strain JAB-1's 193,338 bp plasmid pSHE-CTX-M (NZ_CP022359) that had a length for matches >1 Kb of 30,225 bp. The best match to VREC0693's IncFIB 132,042 bp plasmid was to *Klebsiella pneumoniae* strain Kpn555's 142,858 bp plasmid pKPN-7c3 (NZ_CP015131) that had a length for matches >1 Kb of 98,455 bp. The best match to VREC0693's IncB 88,790 bp plasmid was to *Salmonella enterica* strain ST4/74 was for a 86,908 bp plasmid TY474p2 (NC_017675) that had a length for matches >1 Kb of 77,323 bp. The best match to *bla*$_{\text{CTX-M-15}}$-positive VREC1013's IncFII 89,945 bp plasmid was to *E. coli* strain M19's 11,321 bp plasmid D (NZ_CP010225) that had a length for matches >1 Kb of 5,925 bp. The best match to VREC1073's IncFIA 156,298 bp plasmid was to *Klebsiella pneumoniae* strain SKGH01 84,941 bp plasmid unnamed 3 (NZ_CP015503) that had a length for matches >1 Kb of 39,187 bp. The best match to *bla*$_{\text{CTX-M-27}}$-positive VREC1428's IncFIA plasmid was to *Shigella sonnei* strain 2015C-3566 was for a 55,820 bp plasmid unnamed1(NZ_CP022458) that had a length for matches >1 Kb of 53,995 bp.

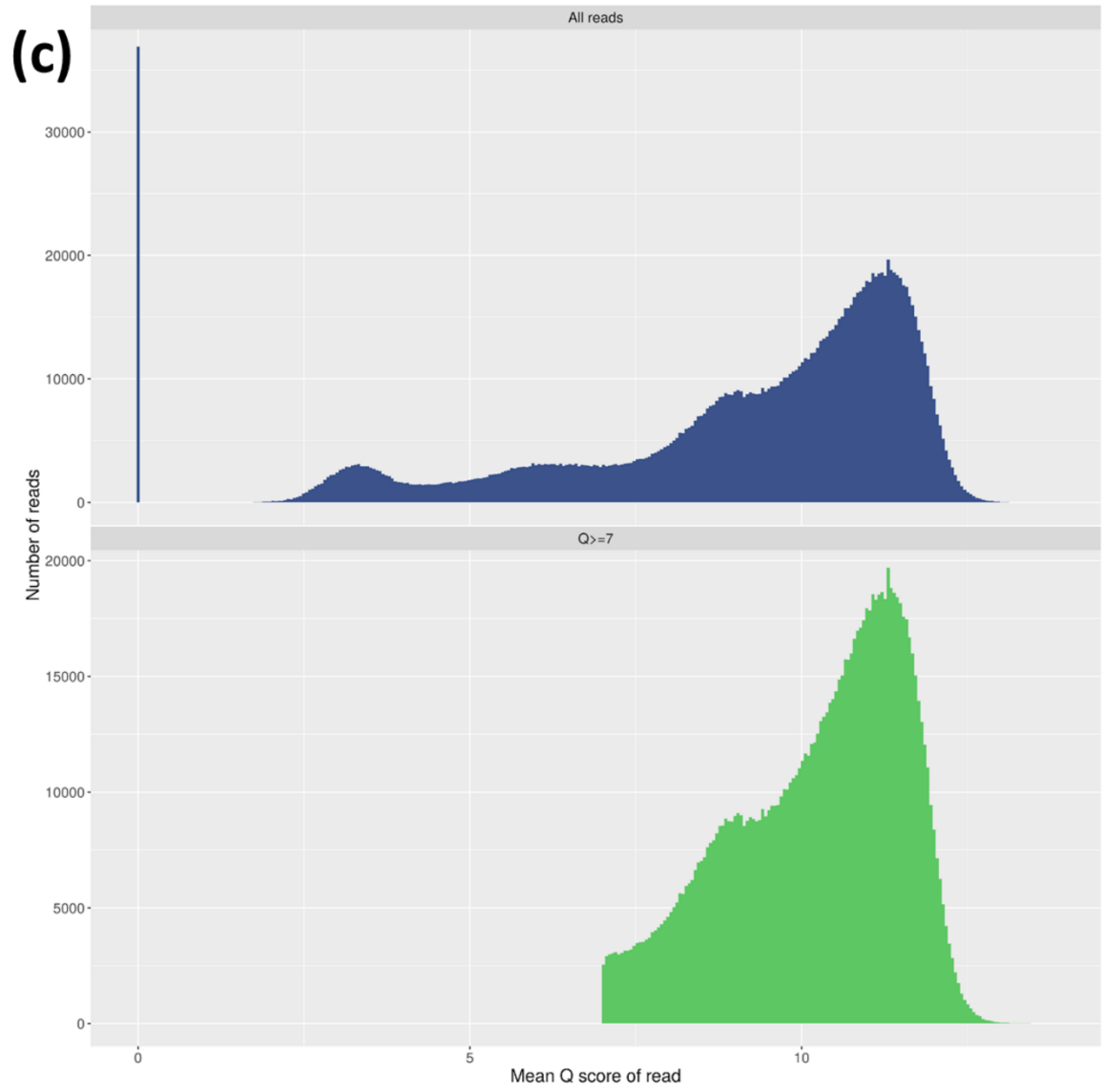
Supplementary Figure 1. Overview of genome assembly using Oxford Nanopore reads to recover plasmids with antibiotic resistance genes and mobile genetic elements (MGEs). Oxford Nanopore fast5 sequences were basecalled and converted to fastq format using Albacore v.2.0 and Guppy v.0.5.1. Forward, reverse and middle adapters were removed using Porechop v.0.2.4. The genomes were assembled using Unicycler v.4.6 (optionally including Illumina short reads for comparison). The probability that the resulting contigs were chromosomal or plasmid-associated was measured using mlplasmids. Contigs were annotated using the Comprehensive Antibiotic Resistance Database (CARD) and Multiple antibiotic Resistance Annotator (MARA) to resolve precise plasmid structure, *bla*$_{\text{CTX-M}}$ gene alleles, copy numbers and their adjacent regions.

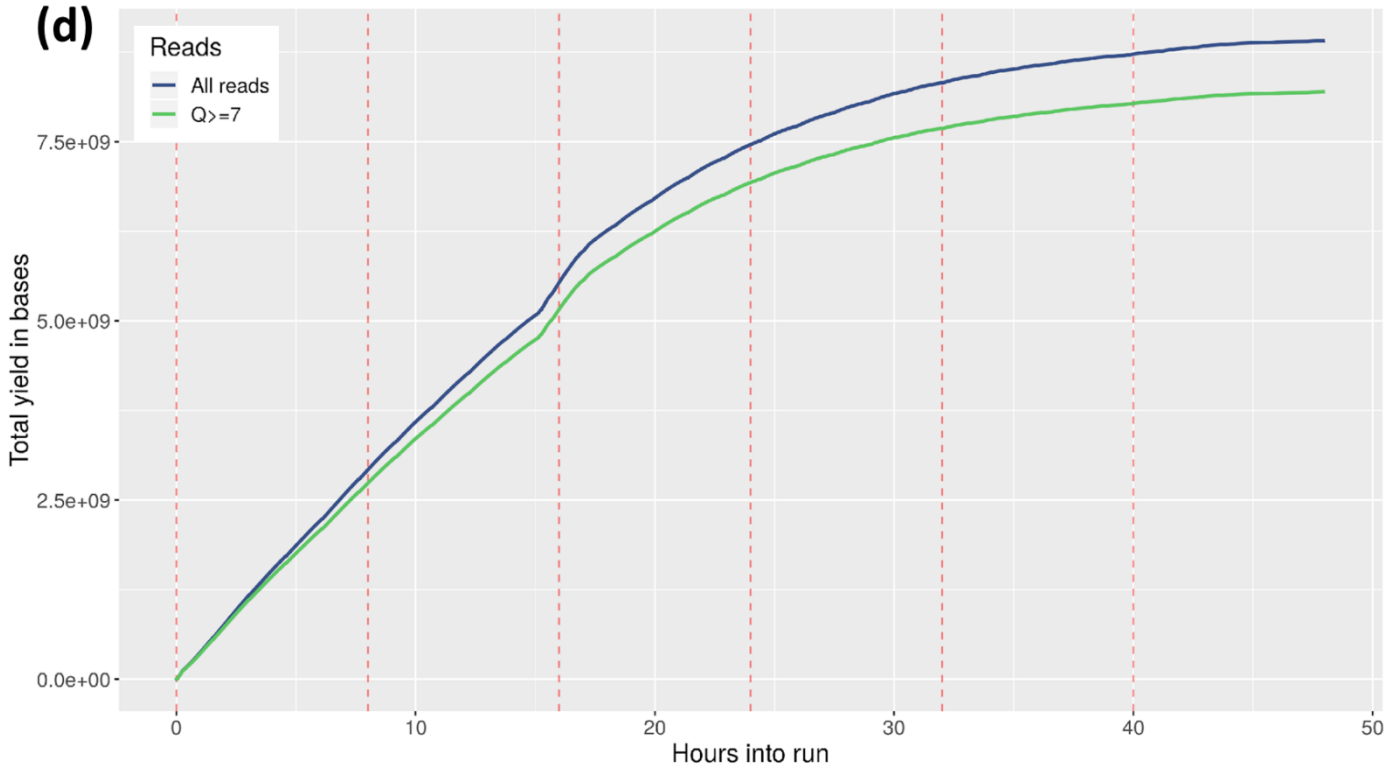


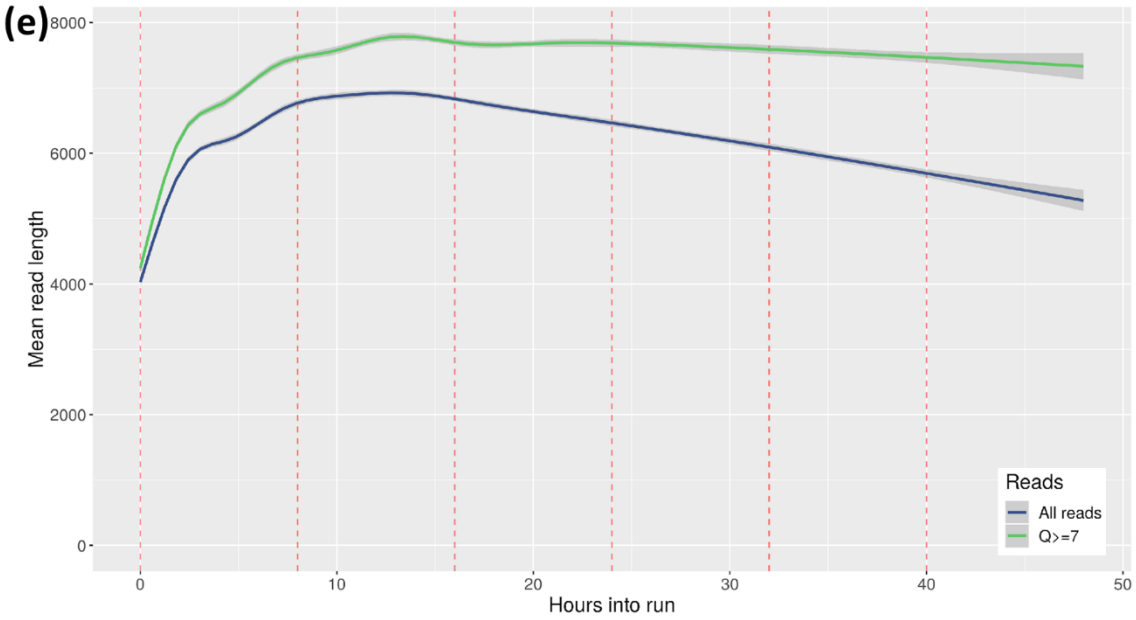
Supplementary Figures 2a-g. Summary plots of the GridION X5 sequencing run for all (blue) and filtered (green) nanopore reads generated using MinIONQC. The graphs in (a) show the read count (y-axis) with the mean and median read length and the number of bases and reads per channel (x-axis), the overall read count (y-axis) vs length (x-axis) in (b) and read count (y-axis) vs the mean Q score (x-axis) in (c). Plots were also drawn to present the total amount of bases called (x-axis; d), the mean read length (x-axis; e) and the mean Q score (x-axis; f) per hour (in their y-axes); the total amount of bases (y-axis) contained in a minimum read length (x-axis) is shown in (g).

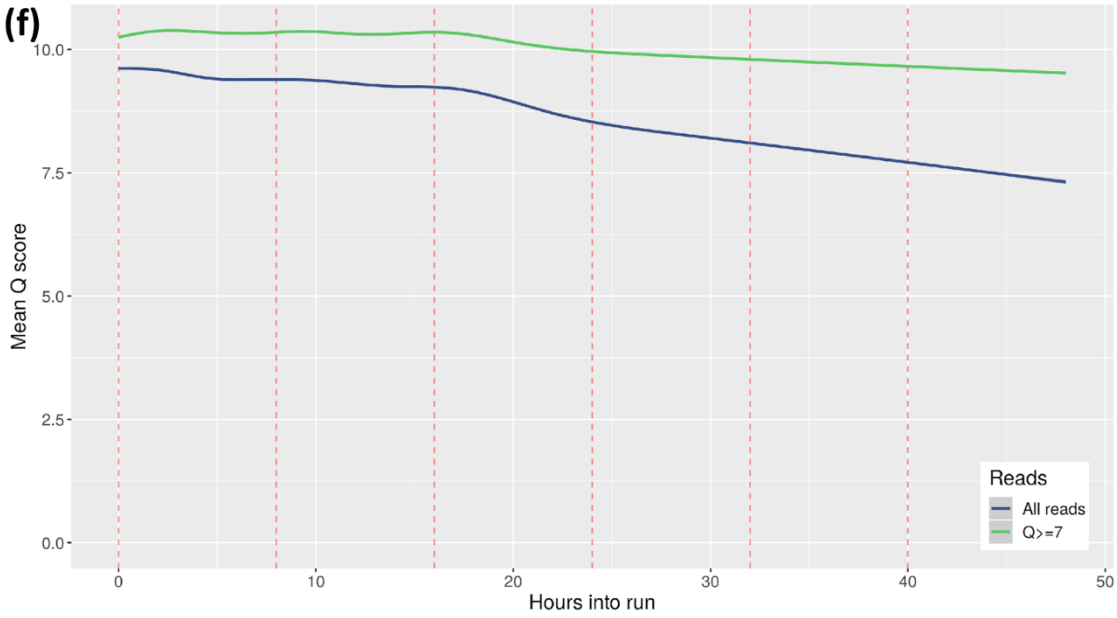


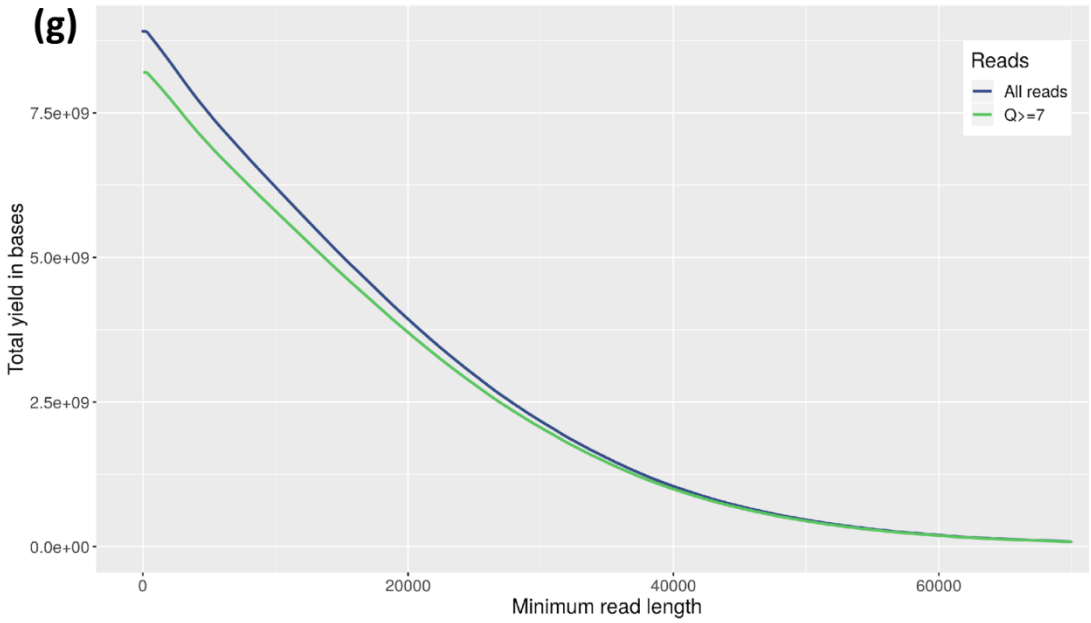



(c)
All reads
30000
20000
10000
0
Number of reads
Q>=7
20000
15000
10000
5000
0
0
5
10
Mean Q score of read

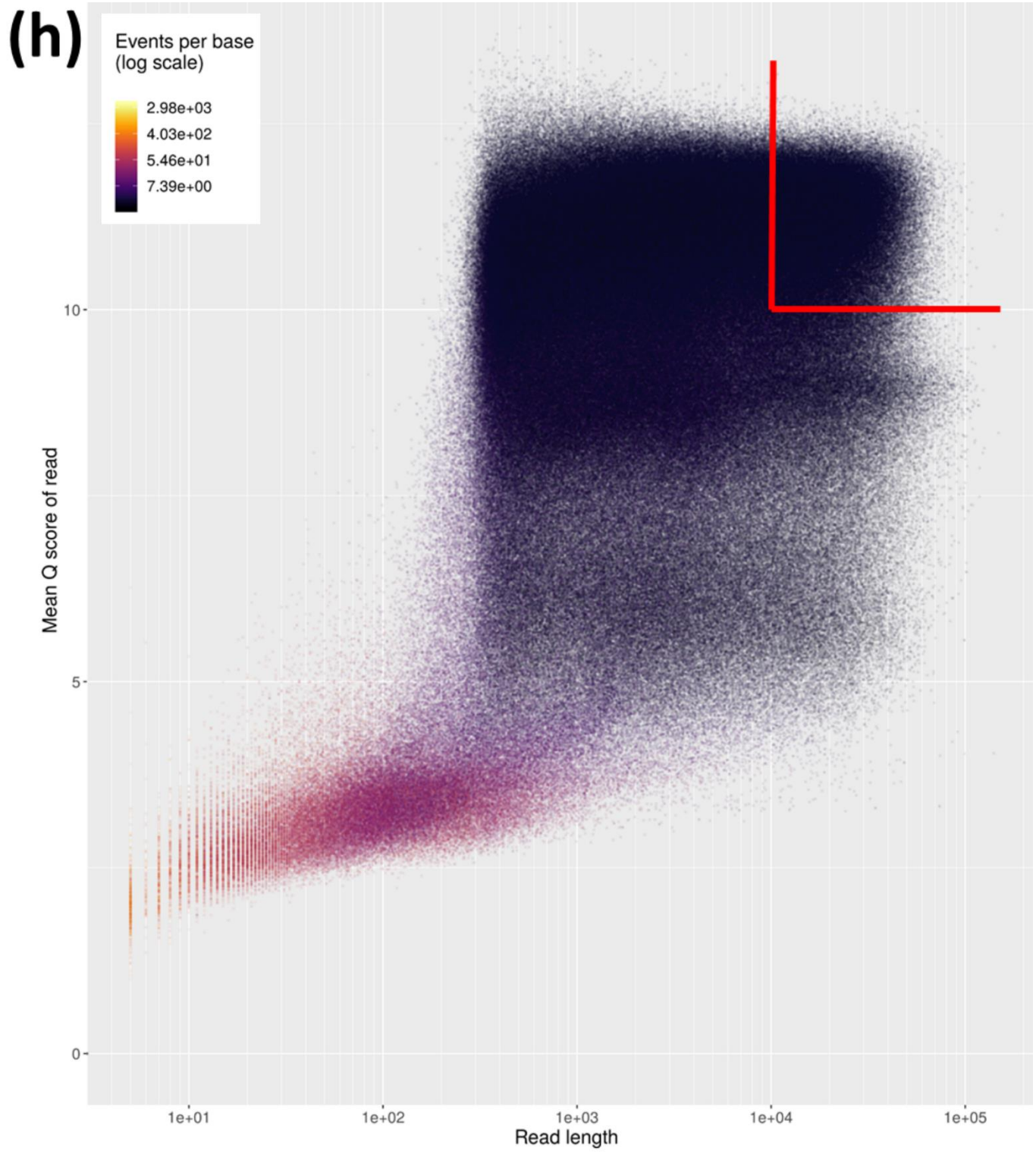

(d)
Reads
All reads
Q>=7
7.5e+09
5.0e+09
2.5e+09
0.0e+00
Total yield in bases
0
10
20
30
40
50
Hours into run


(e)
Mean read length
8000
6000
4000
2000
0
0
10
20
30
40
50
Hours into run
Reads
All reads
Q>=7

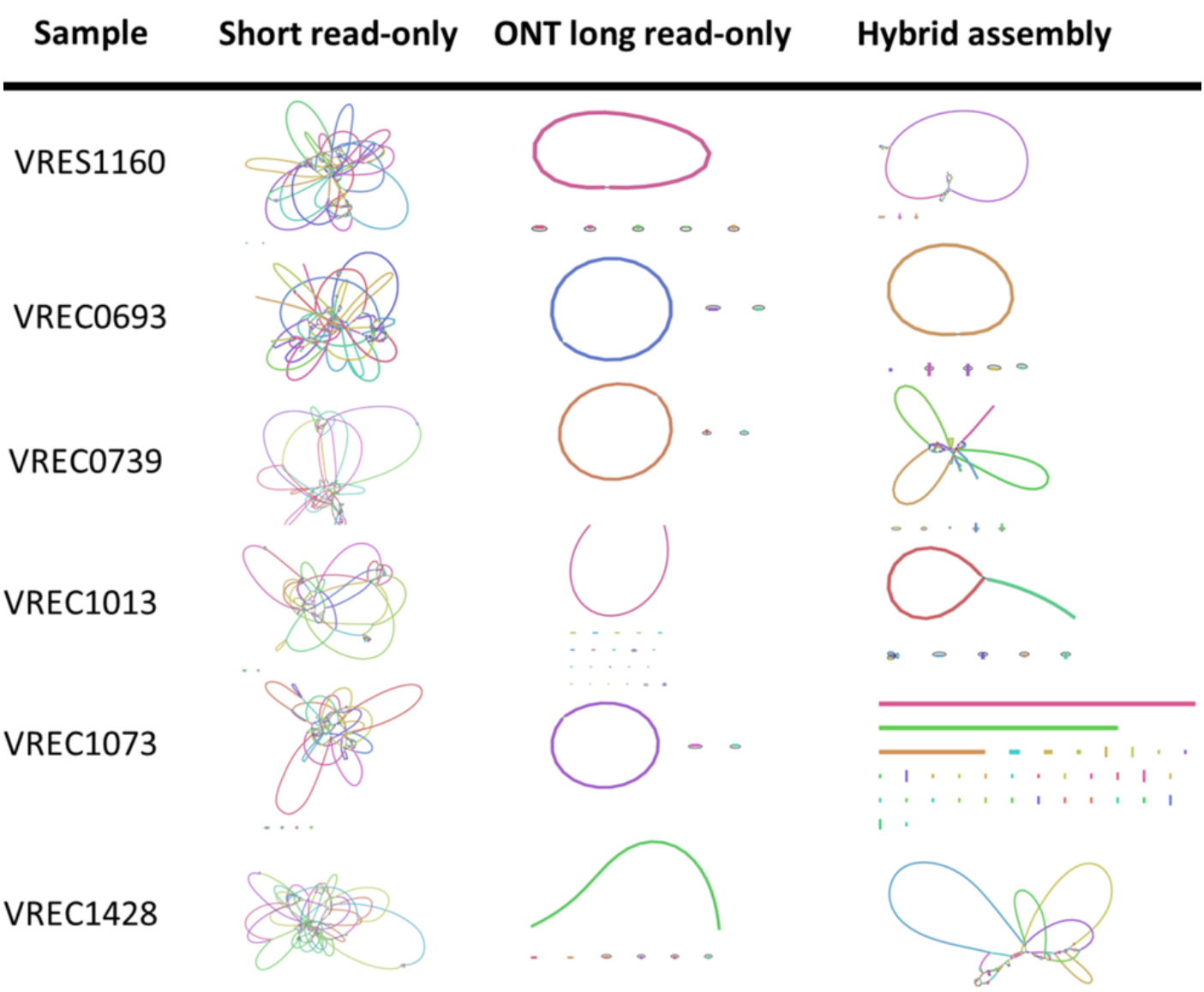

(f)
Mean Q score
10.0
7.5
5.0
2.5
0.0
0
10
20
30
40
50
Hours into run
Reads
All reads
Q>=7

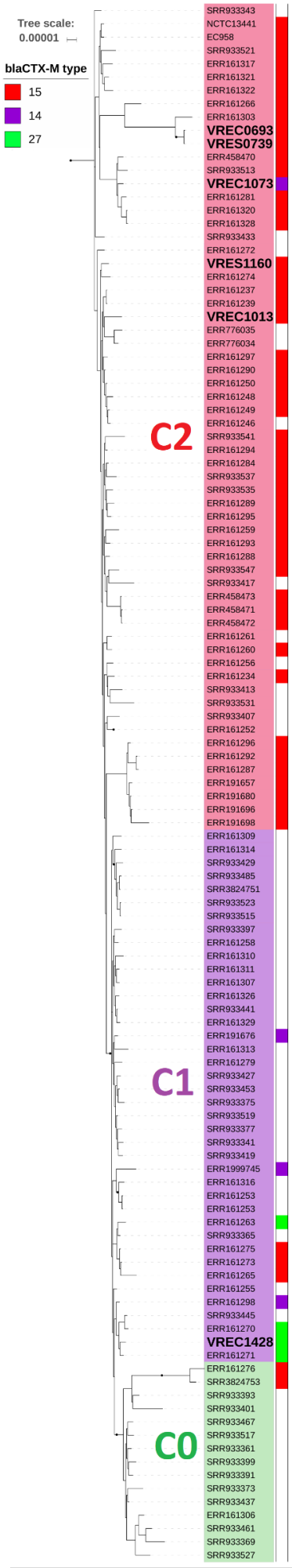

(g)
Total yield in bases
7.5e+09
5.0e+09
2.5e+09
0.0e+00
0
20000
40000
60000
Minimum read length
Reads
All reads
Q>=7

Supplementary Figure 3. Summary of the GridION X5 sequencing run output showing the read length on a log10 scale (x-axis) versus the mean Q score of each read (y-axis) where points are coloured by events per base. The horizontal red line shows reads with lengths > 10 Kb and the vertical red line read with Q scores > 10. Together, this area shows the large number of long high-quality reads generated in this study. This plot emphasises that a high proportion of the bases were accurately called: these were subsequently used for downstream analysis.



Supplementary Figure 4. The assembly graphs of six *E. coli* ST131 genomes showed many connected edges for those created from short Illumina HiSeq reads only (left) but near-complete assemblies for those made with long Oxford Nanopore read-only (centre) and the hybrid assemblies of most of the strains (right). The assemblies were generated with Unicycler v.4.6 and were visualised using Bandage. Circularized contigs indicated complete assemblies.





Supplementary Figure 6. Phylogram of the six ST131 genomes showed that all except VREC1428 were in ST131 subclade C2 (red: VRES1160, VREC1073, VRES0739, VREC0693 and VREC1013). VREC1428 clustered in subclade C1 (purple). No new isolate was in C0 (green). The phylogram was built with RAxML v.8.2.11 and iTOL v4.3 using 3,603 non-recombinant SNPs from Gubbins v.2.3.4 where branch support was performed by 100 bootstrap replicates, and the scale bar indicates the number of substitutions per site. Clade classification was based on phylogenetic analysis by [8] by including the reference NCTC13441, n=63 isolates from [8] and n=56 from [42] with associated classification and *bla*$_{\text{CTX-M}}$ allele data. The right-hand part shows *bla*$_{\text{CTX-M-15}}$ (red), *bla*$_{\text{CTX-M-14}}$ (purple) and *bla*$_{\text{CTX-M-27}}$ alleles (green). The six isolates' names are in large bold text. This mid-pointed rooted phylogeny included reference genome isolates EC958 and NCTC13441 (both in C2) and a clade B isolate as an outgroup (Figure 3). The C2 isolates were mainly *bla*$_{\text{CTX-M-15}}$-positive (48 out of 62, including VRES1160, VRES0739, VREC0693 and VREC1013), bar 13 that were *bla*$_{\text{CTX-M-}}$-negative and one that was *bla*$_{\text{CTX-M-14}}$-positive (VREC1073). The C0 isolates were mainly *bla*$_{\text{CTX-M-15}}$-negative (13 out of 15), as were the C1 (30 out of 40) isolates except for four that were *bla*$_{\text{CTX-M-27}}$-positive, three that were *bla*$_{\text{CTX-M-15}}$-positive and three that were *bla*$_{\text{CTX-M-14}}$-positive.

Supplementary Table 1. Sample collection source, sampling date and sequence read accession numbers.

Strain	Source	Sampling date	Accession numbers		FigShare long read library locations
			Short reads	Long reads	
VRES1160	Faeces	26/08/2015	ERR1878359	ERR3284709	https://ndownloader.figshare.com/files/14039495
VREC0693	Faeces	03/06/2015	ERR2137889	ERR3284704	https://ndownloader.figshare.com/files/14039639
VRES0739	Faeces	05/06/2015	ERR1878196	ERR3284708	https://ndownloader.figshare.com/files/14039354
VREC1013	Faeces	19/08/2015	ERR2138591	ERR3284705	https://ndownloader.figshare.com/files/14039333
VREC1073	Blood	26/08/2015	ERR2138200	ERR3284706	https://ndownloader.figshare.com/files/14039345
VREC1428	Faeces	22/10/2015	ERR2138475	ERR3284707	https://ndownloader.figshare.com/files/14039351

Supplementary Table 2. Contigs were classified as chromosomal or plasmid-derived using the mlplasmids prediction value. Each contig were aligned against CARD to identify the presence/absence of *bla*$_{\text{CTX-M}}$ alleles and their copy numbers. Plasmid types were identified using PlasmidFinder.

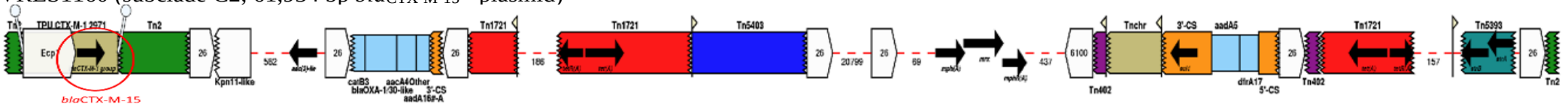
Strain	Prediction	Prediction value (%)	Contig ID	Length (bp)	*bla*$_{\text{CTX-M}}$ allele	*bla*$_{\text{CTX-M}}$ count	Plasmid type	Median Depth	Normalized Depth
VRES1160	Chromosome	98	1	5,126,679			-	258	1.00
	Chromosome	70	2	113,086			-	213	1.00
	Plasmid	70	3	61,934	15	1	IncFIA	282	1.10
	Plasmid	85	4	15,803			ColRNAI	420	1.64
	Plasmid	81	5	5,203			ColRNAI	11	0.04
	Plasmid	83	6	4,096			Col8282	473	1.85
VREC0693	Chromosome	98	1	5,039,909	15	3	-	258	1.00
	Plasmid	61	2	132,042			IncFIB	213	0.83
	Plasmid	60	3	88,790			IncB	282	1.09
VRES0739	Chromosome	98	1	4,797,749			-	171	1.00
	Plasmid	96	2	5,162			Col156	436	2.55
	Plasmid	74	3	4,001			-	303	1.77
VREC1013	Chromosome	97	1	3,699,451			-	300	1.00
	Chromosome	97	2	1,434,037			-	335	1.00
	Plasmid	84	4	89,945	15	1	IncFII	1015	3.27
VREC1073	Chromosome	98	1	5,286,804			-	214	1.00
	Plasmid	68	2	156,298			IncFIA	172	0.80
	Chromosome	60	3	96,056	14	1	-	213	1
VREC1428	Chromosome	98	1	4,924,536			-	126	1.00
	Chromosome	97	2	103,034			-	57	1.00
	Chromosome	96	3	101,160			-	41	1.00
	Plasmid	64	4	92,750	27	1	IncFIA	85	0.67
	Plasmid	92	5	5,147			ColRNAI	168	1.33
	Plasmid	99	6	5,143			Col156	207	1.64
	Plasmid	73	7	4,649			ColRNAI	239	1.90

Supplementary Table 3. Comparison of short read-only, long read-only and hybrid genome assemblies generated using the conservative, normal and bold modes of Unicycler v.04.6. Assemblies were assessed according to their total length, number of contigs produced, N50 (bp), numbers of mismatches per 100 Kb and numbers of indels per 100 Kb.

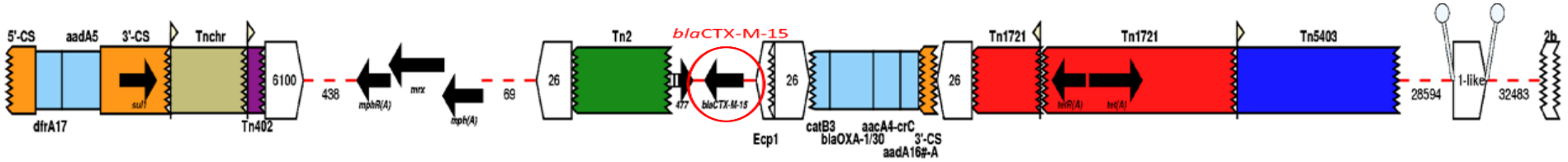
Assembly	Mode	Metric	VRES1160	VREC0693	VRES0739	VREC1013	VREC1073	VREC1428
Short read-only	Conservative	Total length (bp)	5,142,342	5,146,205	5,181,497	5,208,807	4,967,093	5,375,468
		Number of contigs	168	159	200	148	117	230
		N50 (bp)	124,175	132,865	138,725	134,439	157,528	135,303
		#mismatches / 100 Kb	1.32	1.32	65.4	1.5	285.81	0.69
		#indels / 100 Kb	0.06	0.02	1.84	0.08	261.91	0.04
	Normal	Total length (bp)	5,158,728	5,171,710	5,227,751	5,240,888	4,989,316	5,416,180
		Number of contigs	110	106	123	94	76	148
		N50 (bp)	206,138	190,908	213,071	189,184	222,158	170,443
		#mismatches / 100 Kb	4.64	0.93	69.86	4.25	284.14	2.96
		#indels / 100 Kb	0.21	0.14	2.33	0.36	262.1	0.04
	Bold	Total length (bp)	5,159,662	5,163,846	5,207,686	5,226,735	4,977,746	5,411,973
		Number of contigs	124	120	146	108	86	140
		N50 (bp)	206,044	190,808	212,979	190,412	222,051	184,466
		#mismatches / 100 Kb	3.07	1.78	67.11	1.96	287.37	2.03
		#indels / 100 Kb	0.16	0.06	2.03	0.13	262.26	0.11
Long read-only	Conservative	Total length (bp)	5,326,801	5,260,741	4,806,912	6,307,464	5,539,158	5,236,419
		Number of contigs	6	3	3	22	3	7
		N50 (bp)	5,126,679	5,039,909	4,797,749	5,073,008	5,286,804	4,924,536
		#mismatches / 100 Kb	276.23	241.39	2,772.51	344.5	0	332.79
		#indels / 100 Kb	252.29	264.7	265	306.03	0	289.71
	Normal	Total length (bp)	5,326,801	5,260,741	4,806,912	6,307,464	5,539,158	5,236,419
		Number of contigs	6	3	3	22	3	7
		N50 (bp)	5,126,679	5,039,909	4,797,749	5,073,008	5,286,804	4,924,536
		#mismatches / 100 Kb	276.23	241.39	2772.51	344.5	0	332.79
		#indels / 100 Kb	252.29	264.7	265	306.03	0	289.71
	Bold	Total length (bp)	5,326,801	5,260,741	4,806,912	6,307,464	5,539,158	5,236,419
		Number of contigs	6	3	3	22	2	7
		N50 (bp)	5,126,679	5,039,909	4,797,749	5,073,008	5,286,804	4,924,536
		#mismatches / 100 Kb	276.23	241.39	2772.51	344.5	0	332.79
		#indels / 100 Kb	252.29	264.7	265	306.03	0	289.71
Hybrid	Conservative	Total length (bp)	5,272,824	5,275,251	5,215,332	5,323,049	5,055,625	5,492,517
		Number of contigs	52	6	191	34	51	107
		N50 (bp)	1,444,640	5,048,264	426,378	2,673,977	1,423,856	749,550
		#mismatches / 100 Kb	1.63	242.24	2,764.2	2.04	285.57	3.7
		#indels / 100 Kb	0.32	265.38	263.44	0.09	263.18	0.02
	Normal	Total length (bp)	5,276,305	5,275,251	5,291,108	5,327,833	5,098,966	5,516,886
		Number of contigs	42	6	110	33	44	74
		N50 (bp)	1,746,191	5,048,264	72,0730	2,675,388	1,762,353	1,243,293
		#mismatches / 100 Kb	1.56	242.24	44.59	2.28	284.11	1.65
		#indels / 100 Kb	0.28	265.38	4.07	0.13	266.82	0.02
	Bold	Total length (bp)	5,293,427	5,275,251	5,267,003	5,223,433	5,115,410	5,550,270
		Number of contigs	23	6	32	3	22	47
		N50 (bp)	3,801,465	5,048,264	1,222,073	3,699,451	4,958,323	1,266,683
		#mismatches / 100 Kb	271.47	242.24	2,770.38	321.64	283.97	296.99
		#indels / 100 Kb	252.55	265.38	264.11	268.47	268.27	268.29

Supplementary Figure 5. The contigs from the most optimal assembly mode of Unicycler v.4.6 of five out of six *E. coli* ST131 samples were identified as chromosomal or plasmid-derived using mlplasmids. These were annotated with *bla*$_{\text{CTX-M}}$ genes and their genetic flanking context using Galileo™ AMR based on the Multiple Antibiotic Resistance Annotator (MARA) and database [35]; all *bla*$_{\text{CTX-M}}$ variants are labelled accordingly and encircled in red (*bla*$_{\text{CTX-M-15}}$), purple (*bla*$_{\text{CTX-M-14}}$) or green (*bla*$_{\text{CTX-M-27}}$). The definition of the other elements are listed at https://galileoamr.arcbio.com/mara/feature/list. The long VREC0693 chromosome is split into two parts so that the gene annotation is visible.

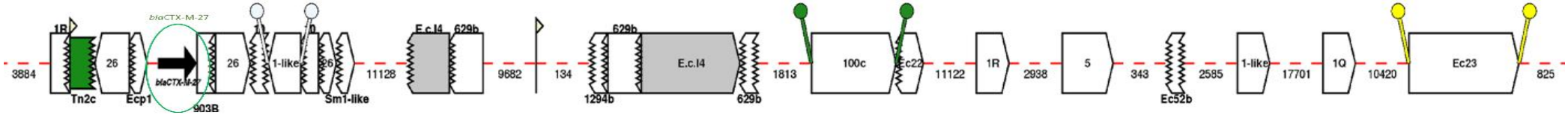
VRES1160 (subclade C2, 61,934 bp *bla*$_{\text{CTX-M-15}}$+ plasmid)



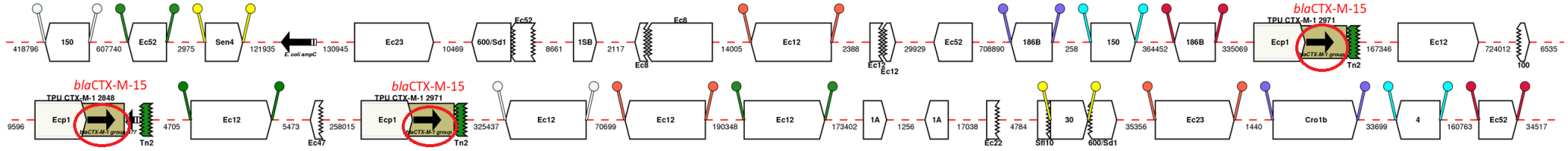
VREC1013 (subclade C2, 89,945 bp *bla*$_{\text{CTX-M-15}}$+ plasmid)



VREC1428 (subclade C1, 92,750 bp *bla*$_{\text{CTX-M-27}}$+ plasmid)



VREC0693 (subclade C2, 5,039,909 bp *bla*$_{\text{CTX-M-15}}$+ chromosome with 3 distinct *bla*$_{\text{CTX-M-15}}$ genes in red - single chromosome is split below for visualisation)



VREC1073 (subclade C2, 96,056 bp *bla*$_{\text{CTX-M-14}}$+)