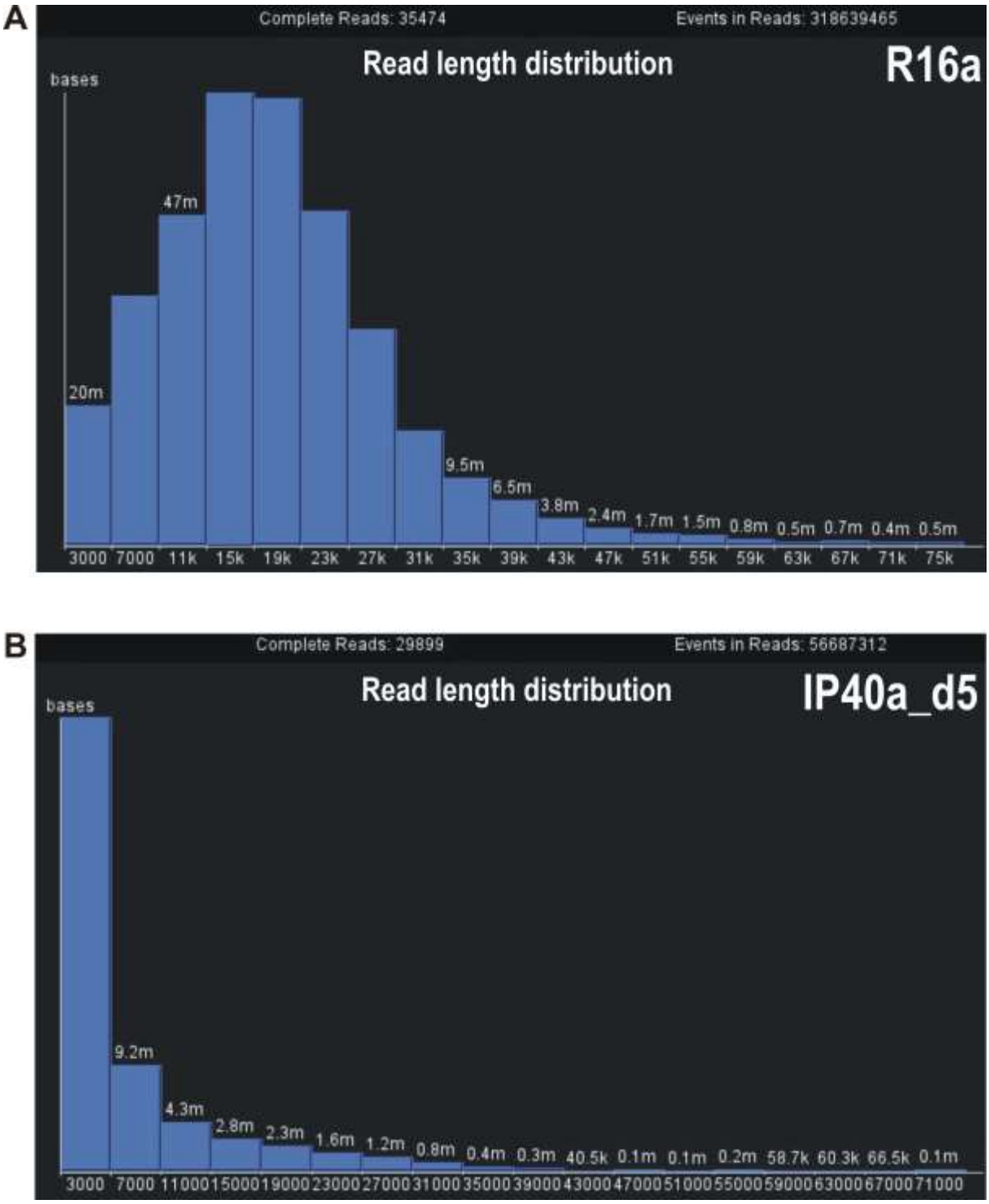

FIG S1 MinION read-length histograms from sequencing of R16a (A) and IP40a_d5 deletion derivative (B). The height of each column shows the cumulative number of bases obtained from reads falling into the particular size range.

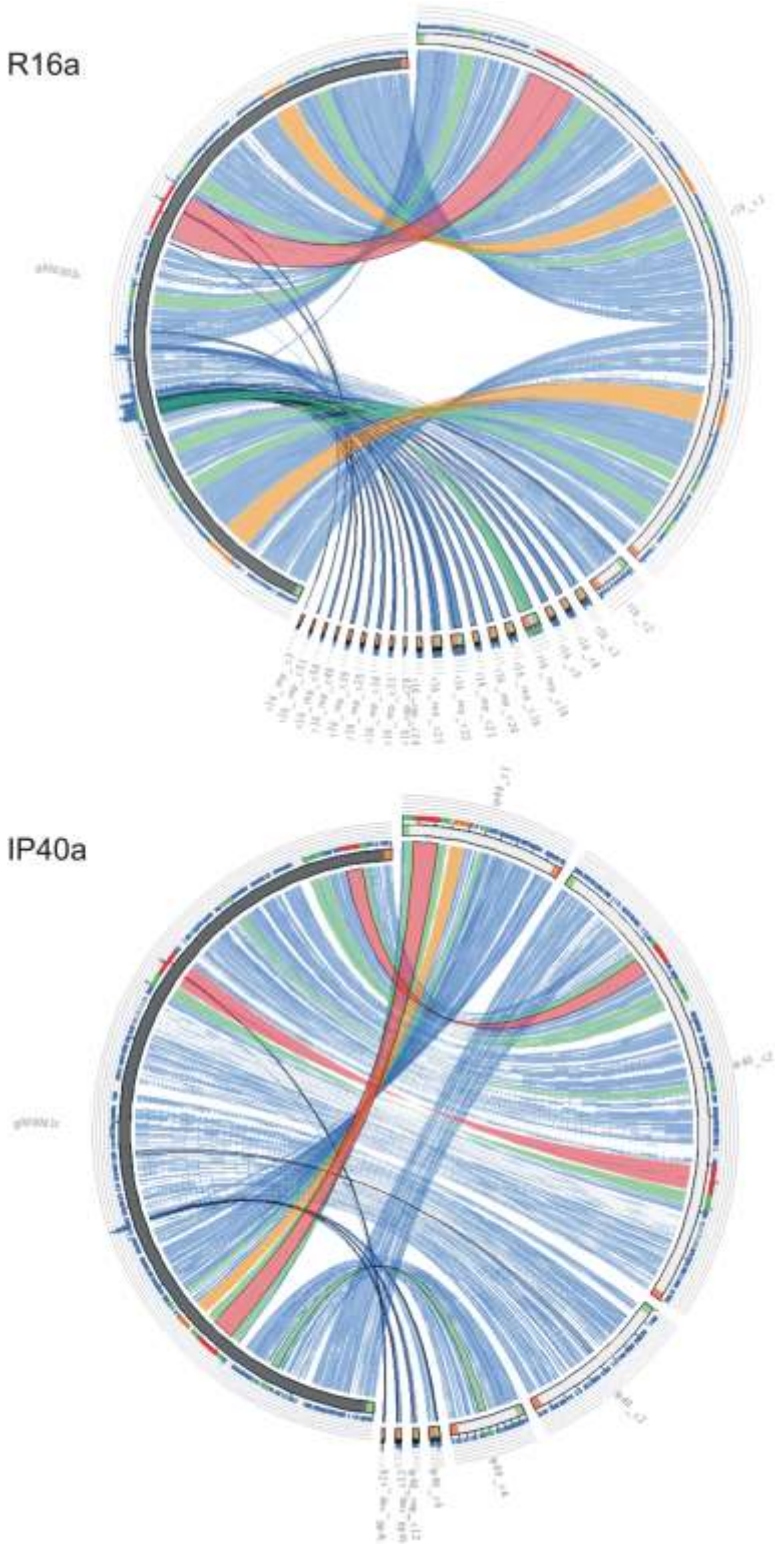

FIG S2 Alignments of MIRA-contigs to the single Miniasm-contigs assembled from R16a and IP40a MinION reads. The Miniasm-contigs (g00003c and g00001c) are shown as dark grey, Mira-contigs are blank (available at http://emboss.abc.hu/minionarticle/). Ribbons represent the local alignments produced by BLAST. Colors correspond to the alignment bit scores in the four quartiles: red, 75-100%; orange, 50-75%; green, 25-50%; blue, <25% of the maximum bit score. Green-to-red direction of ideograms shows the 5'-3' orientation of contigs. Ribbons are inverted if reverse complementary sequence are aligned. Diagram was generated using Circoletto.(1)

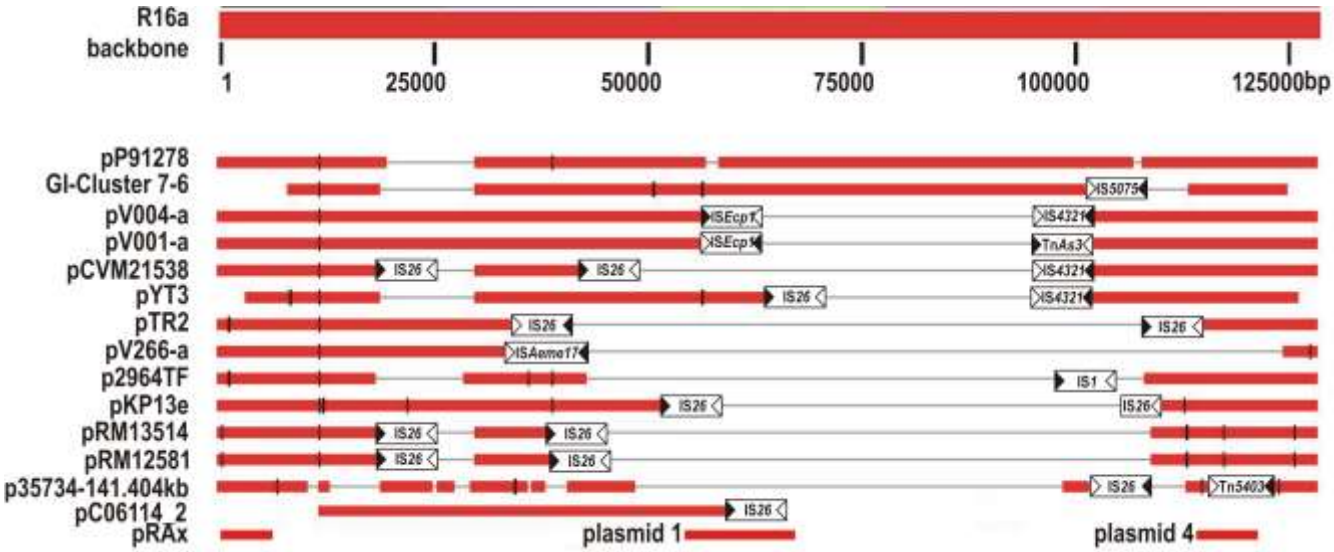

FIG S3 Correlation between the endpoints of backbone segments and the location of IS elements in 16 IncA/C family members possessing highly incomplete backbones. The alignment was generated by Blast using R16a backbone as query sequence, which was generated by removing the sequence of ARI_{R16a}, GIsul2 and one copy of their flanking TDs from the reference R16a sequence (KX156773). Only the ISs located adjacent to the backbone segments are indicated.

Table S1 Oligonucleotide primers used.

Name	Sequence (5'→3')	References
IP40_c1_5out	gcgctcaaacagtcagatcag	this work
IP40_c4_5out	tcatccaggtcatcgagcgtg	this work
IP40_c3_5out	gtttccgtttgcagcttcacg	this work
IP40_c2_3out	gcagctttgtgttgctcaaac	this work
IP40_c2_5out	ttgcccgccgcaaccatcttc	this work
IP40_c4_3uot	gattgatgccgataccatgctg	this work
IS150Rout	cgggatccgaatactacaacagcagaagaattag	(2)
R16a_c2_5out	tgctgtctggtcagtccatgc	this work
R16a_c1_5out	ttgatggtctgcaaggtacttc	this work
amp5	ttctgcagcaacgatcaaggcgagttacatg	this work
pBRBgl	ttaccatctggccccagtgctgc	(3)
R16a_c2_3out	cgtggagcgtatcggcggaag	this work
R16a_c3_5out	ggtgttctcctcatccggatcg	this work
R16a_c3_3out	aatcgcggcctcgagcaagac	this work
R16a_c1_3out	cgatattcttcgatctagcacagg	this work
parB_BamHIrev	ataggatccttagccttcgacactcaggag	this work
parB_Ndefor	ggccatatggcattgaacaatttaaaaggcctg	this work
IS15DII_3out	gcgatcatggcaaactgaaacg	this work
catfor	aaatcactggatataccaccgttg	(4)
catrev	gtactgttgtaattcattaagc	this work
R55_dfloseqfor	ccaagtcagcagacaagtaagc	this work
R55_dfloseqrev	ccgcatgccgaaaattcagatgc	this work
R55_aadoxafor	ttctcgagtaggccgcatggacacaacgc	this work
R55_aadoxarev	ttctcgagtttatcgcgctgcgtccgagttgac	this work
R55_aadbrevNP	aactgcagcggccgcttaggccgcatatcgcgacctg	this work
SmRforSmP	cgctgcagcccggggttgccgggtgacgcac	this work
R55_dTn6186seqfor	aactgcaggcgttttgcagcgttc	this work
R55_dTn6186seqrev	tagtcgacagatttagaccatcatgcaacg	this work
IS5075_5out	ctaactcttccagcttgcgtgc	this work
IP40Tpout	gaaaactcaagacttcgtaatcgg	this work
IS15DI_5out	catctcctgcagctcacggtaac	this work
cat5	gtatcaacagggacaccaggattta	(3)
R55_T1rev3	ttctgcagcttcaaaggtcgctcttacatc	this work

References

1. **Darzentas N**. 2010. Circoletto: visualizing sequence similarity with Circos. Bioinformatics **26**:2620–2621.
2. **Szeverényi I**, **Nagy Z**, **Farkas T**, **Olasz F**, **Kiss J**. 2003. Detection and analysis of transpositionally active head-to-tail dimers in three additional Escherichia coli IS elements. Microbiology **149**:1297–1310.
3. **Kiss J**, **Nagy Z**, **Tóth G**, **Kiss GB**, **Jakab J**, **Chandler M**, **Olasz F**. 2007. Transposition and target specificity of the typical IS30 family element IS1655 from Neisseria meningitidis. Mol Microbiol **63**:1731–1747.
4. **Szabó M**, **Kiss J**, **Nagy Z**, **Chandler M**, **Olasz F**. 2008. Sub-terminal Sequences Modulating IS30 Transposition in Vivo and in Vitro. J Mol Biol **375**:337–352.

Table S2 List of IncA/C family plasmids and the major characteristics applied in classification (data as of 11.08.2016).

Table S3 Target site of GIsul2 insertions

Host strain:	location of GIsul2	start-end positions of GIsul2	direct repeat
P. stuartii ATCC 33672 (CP008920.1)	3' end of *gua*A	3629964→3614509 (complement)	ggga GIsul2 ggga
Escherichia coli PMV-1 pHUSEC411-like plasmid (HG428756.1)	intergenic region downstream of *tol*A	30675→46130	agg-a GIsul2 ggga
Sphingopyxis granuli strain TFA (CP012199.1)	3' end of *gua*A	1447616→1432161 (complement)	ggga GIsul2 ggga
Morganella morganii strain FDAARGOS_172 (CP014026.1)	3' end of *gua*A	1103381→1118836	ggga GIsul2 ggga
Achromobacter xylosoxidans genome assembly NCTC10807 (LN831029.1)	3' end of *gua*A_3	3809594→3794139 (complement)	ggga GIsul2 ggga
Escherichia coli strain 6409 (CP010371.1)	3' end of *gua*A	2706923→2691468 (complement)	ggga GIsul2 ggga
Morganella morganii strain M203 plasmid R485 (HE577112.1)	inside *top*B	38228→53683	ggga GIsul2 ggg-t
Shigella flexneri G1663 (CP007037.1)	3' end of *gua*A	2617889→2602434 (complement)	ggga GIsul2 ggga
Shigella flexneri 2a str. 2457T (AE014073.1)	3' end of *gua*A	2614002→2598551 (complement)	ggga GIsul2 ggga
Enterobacter cloacae subsp. cloacae ATCC 13047 (CP001918.1)	3' end of *gua*A	3927442→3902209 (complement)	ggga GIsul2 (+9774 bp Tn) ggga
R16a (KX156773)	3' end of a hypothetical gene	29951→45406	ggga GIsul2 ggga
IP40a (KX156772)	3' end of a hypothetical gene	31276→48177	ggga GIsul2 (+1443 bp IS*150)* ggga

Table S4 Origin of the deletion derivatives

Origin	Phenotype	Passage	Frequency of the phenotype	Isolates in the phenotype category/total colonies tested	Structurally identified isolates	Name of deletion derivative
R16a	$Km^{S}Ap^{R}$	1	2.8×10^{-3}	1/349	1	R16a_d1a
	$Km^{S}Ap^{R}$	5	3.5×10^{-3}	3/854	2	R16a_d1b/1,2
	$Km^{S}Ap^{R}$	5	1.8×10^{-3}	1/549	1	R16a_d1c
	$Km^{S}Ap^{R}$	4	9.0×10^{-3}	1/111	1	R16a_d2
	$Km^{S}Ap^{R}$	3	3.8×10^{-2}	3/80	1	R16a_d3
IP40a	$Km^{S}Ap^{S}$	5	3.8×10^{-3}	2/520	1	IP40a_d5
	$Km^{S}Ap^{S}$	5	2.2×10^{-3}	1/451	1	IP40a_d8
R55	$Km^{S}Gm^{S}Ap^{S}Flo^{R}Cm^{R}$	5	9.6×10^{-3}	5/519	4	R55_d11a/1-4
	$Km^{S}Gm^{S}Ap^{S}Flo^{R}Cm^{R}$	5	6.9×10^{-3}	3/583	3	R55_d11b/1-3[a]
	$Km^{S}Gm^{S}Ap^{S}Flo^{R}Cm^{R}$	5	5.4×10^{-3}	3/557	3	R55_d11c/1-3
	$Km^{S}Gm^{S}Ap^{S}Flo^{R}Cm^{R}$	1	6.5×10^{-3}	1/155	1	R55_d11d
	$Km^{S}Gm^{S}Ap^{S}Flo^{R}Cm^{R}$	5	6.9×10^{-3}	1/583	1	R55_d20[a]

[a] The R55_d20 and the three R55_d11b isolates derived from the same experiment.

Text S1 Supplementary Materials and Methods

Gap closure and structural verification of complete plasmid sequences

The gap in R16a sequence was sealed by sequencing the amplicon obtained with primers R16a_c2_3out–R16a_c3_5out. The assembly of ARI_{R16a} region was verified by PCRs using combinations of the following primers: R16a_c2_5out, R16a_c1_5out, amp5, pBRBgl, R16a_c3_3out and R16a_c1_3out.

For completion of IP40a sequence three gaps were closed by PCRs. Amplicons were generated and sequenced with primer pairs IP40_c1_5out–IP40_c4_5out; IP40_c3_5out–IP40_c2_3out and IP40_c2_5out–IP40_c4_3out. Amplicon obtained with primers IP40_c2_5out and IP40_c4_3out was also sequenced with IS150Rout.

Verification of deletions

Plasmid retention was first proved for all clones that lost resistance markers by amplifying parB gene of IncA/C plasmids with primers parB_Ndefor–parB_BamHIrev.

R16a_d1-type deletions were verified using R16a_c2_3out–R16a_c1_3out for PCR, and R16a_c2_3out, IS15DII3out for amplicon sequencing. Deletion region in R16a_d2 was amplified with R16a_c2_3out–R55_T1rev3 and sequenced with IS15DII3out. R16a_d3 structure was proved by PCR with primers amp5–R16a_c1_3out, and analysis of the 4.55 kb amplicon by *Pst*I, *Eco*RV and *Bam*HI digestions.

R55 deletions were pre-screened for the presence of different resistance regions by PCRs using primer pairs catfor–catrev, R55dfloseqfor–R55dfloseqrev, R55aadoxafor–R55aadoxarev and R55_aadbrevNP–SmRforSmP. R55_d11-type deletions were then proved by PCRs carried out with primers R55_dTn6186seqfor–R55-dTn6186seqrev and sequencing the amplicons with primer IS5075_5out.

Deletions identified by annealed reads from MinION sequencing were also verified by PCRs and sequencing the appropriate amplicons as follows: IP40a_d5 and IP40a_d8 were amplified with primers IP40Tpout – IS15DI5out and the amplicons were sequenced with IP40Tpout, while R55_d20 was amplified with primers aadboxarev – cat5 and the amplicon was sequenced with aadboxarev.