Table S1. Primers used for the detection of the *iee* gene and IS elements

Target	Primer name	Sequence	Expected amplicon size (bp)
iee	ieeF4	GATAAACGATTTGCCGGGACGGAATG	280
	ieeR4	GTTTACCTCCCCCGATAATACCAATAC	
IS*629*	IS629-TF1	ACTAAAAATACTCGTTTTTCCCCCGAAG	1,208
	IS629-TR1	GGCTGCCAGATCATCGTTTCCGATG	
IS*911*	IS911-TF1	AAAAAAAGAAATTTCAGCGCAGAGTTTAAA	1,141
	IS911-TR1	GAGTTTTTTCCAGTATCGGTTTTCCGAT	
IS*3*	IS3-TF1	ACAAAAACAGTATCAACCAGTAAAAAACCC	1,157
	IS3-TR1	AGCGAGGTTCTTGTTTTCAAATTGTTCC	
IS*2*	IS2-TF1	ATTGATGTCTTAGGGCCGGAGAAACG	1,223
	IS2-TR1	TATTTCCAGACATCTGTTATCACTTAACC	
IS*1*	IS1-TF1	GCTTCTGTTTCTATCAGCTGTCCCTC	692
	IS1-TR1	TTGATAGTGTTTTATGTTCAGATAATGCCC	
IS*4*	IS4-TF1	CACATTGGACAGGCTCTTGATCTGGT	1,323
	IS4-TR1	AGCAACTGACTGGCTCTTTTTCGGGG	
IS*5*	IS5-TF1	AGTCATCAACTTACCTTCGCCGACAG	975
	IS5-TR1	GTGAGATCTCTCCCACTGACGTATCA	
IS*26*	IS26-TF1	AACCCATTCAAAGGCCGGCATTTTCAG	699
	IS26-TR1	CATTTCAAAAACTCTGCTTACCAGGCG	
IS*30*	IS30-TF1	AGACGAACTATTACAGCAGAGGAAAAAG	1,146
	IS30-TR1	ATCTGTCAATGCAACACCCCTTTCAATT	
IS*621*	IS621-TF1	GAACATGAACTTCATTATATCGGTATCGAC	975
	IS621-TR1	CGCCGCTACCGGATTATGCCGTGA	

Table S2. Newly-designed primers used for the analysis of the genomic structures of *iee*-containing elements

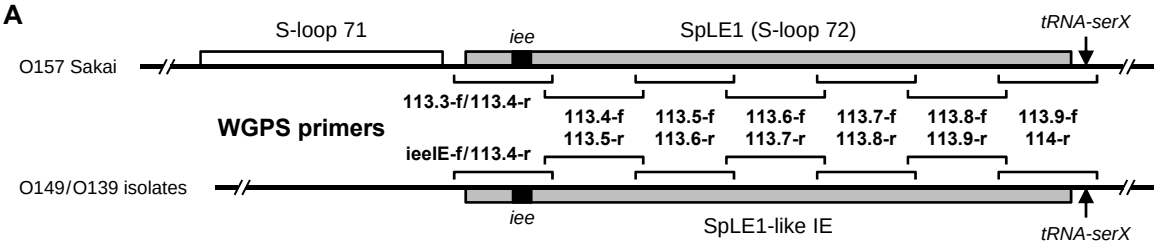
Primer name	Sequence	Primer coordinates	Accession number
ieeIE-f	CCAGTTGTCGAACCCCGGTCGGGGCTTCTC	1187053−1187082	CP002729
113.9-r2	GTTGGTTTTGTAATTCCAGCTCAACC	1277823−1277798	CP002729
IE0231-f	TCATCCAGGACAACCTGAAAAAACCGC	987783−987809	CP002729
IE0231-r	GGCGAAGAGAAAGAACGAGTAAAAGGTC	988755−988782	CP002729

Table S3. Primers used for RETS-PCR to analyze the junctions of SpLE1-like IE in E0231

Target	Primer name	Sequence[a]	Step of RETS-PCR
5’ region	E0231J-R1	GCCATTAGTCTCTCGCTTCACNNNNNNNNN	First
	E0231J-R 2	GCCATTAGTCTCTCGCTTCAC	Second
	E0231J-R 3	CTAAGAATACATTAGATAATATCGGACAAC	Third
3’ region	E0231J-F1	CGGGGCATATAAACCGGTTTCNNNNNNNNN	First
	E0231J-F 2	CGGGGCATATAAACCGGTTTC	Second
	E0231J-F 3	TTCAGATGGCATACCCGATGGCATAC	Third

[a] N, a mixture of A, G, C, and T.

A



B

Strain	Source	Serotype	*iee*	ieeIE-f 113.4-r	113.4-f 113.5-r	113.5-f 113.6-r	113.6-f 113.7-r	113.7-f 113.8-r	113.8-f 113.9-r	113.9-f 114-r
				PCR primers						
E0092, E0098, E0126	Ibaraki	O149	+	15 kb	15 kb	13 kb	14 kb	12 kb	11 kb	15 kb
E0221–E0230, E0232, E0233	Fukuoka	O149	+	15 kb	15 kb	13 kb	14 kb	12 kb	11 kb	15 kb
E0231	Fukuoka	O149	+		16 kb	13 kb	16 kb	12 kb	*	
E0129	Ibaraki	O149	–							
E0124, E0131	Ibaraki	O139	+	15 kb	16 kb	13 kb	14 kb	12 kb	11 kb	15 kb
E0043–E0059	Tokyo	O139	+	15 kb	16 kb	13 kb	14 kb	12 kb	11 kb	15 kb
E0217–E0220	Chiba	O139	+	15 kb	16 kb	13 kb	14 kb	12 kb	11 kb	15 kb
E0095, E0111, E0115, E0128	Ibaraki	O139	–							
E0234, E0237, E0241, E0242, E0250, E0251, E0254	Fukuoka	O139	–							
E0001–E0008	Miyazaki	O139	–							
E0009–E0011, E0017–E0027	Iwate	O139	–							

Figure S1. Genomic localization of *iee* in the ETEC O139 and O149 strains. (A) Schematic representation of the PCR scanning analysis of the SpLE1-like IEs in the ETEC O139 and O149 strains. The *iee* gene (black rectangle) is located in SpLE1 or SpLE1-like IEs (gray rectangles) in the O157 Sakai and the ETEC O139/O149 strains, respectively. The names and positions of the WGPS primers used to amplify each segment are indicated. To amplify the left regions of the SpLE1-like IEs, primer ieeIE-f was designed and used as a substitute for primer 113.3-f because the regions located upstream of the SpLE1-like IEs in the O139 and O149 ETEC strains differed in sequence from that of the SpLE1 in O157. (B) Summary of the results of the PCR scanning analysis. The strains were grouped according to their PCR amplification patterns and other strain features (i.e., source, serotype, and possession of *iee*). The segments that were not amplified are indicated by black rectangles, and those that were amplified are indicated by open rectangles with the size of each amplicon. As indicated by an asterisk, no amplicon was obtained using the primer pair 113.8-f/113.9-r in strain E0231; however, this region was amplified using the newly designed primer 113.9-r2 as a substitute for 113.9-r.

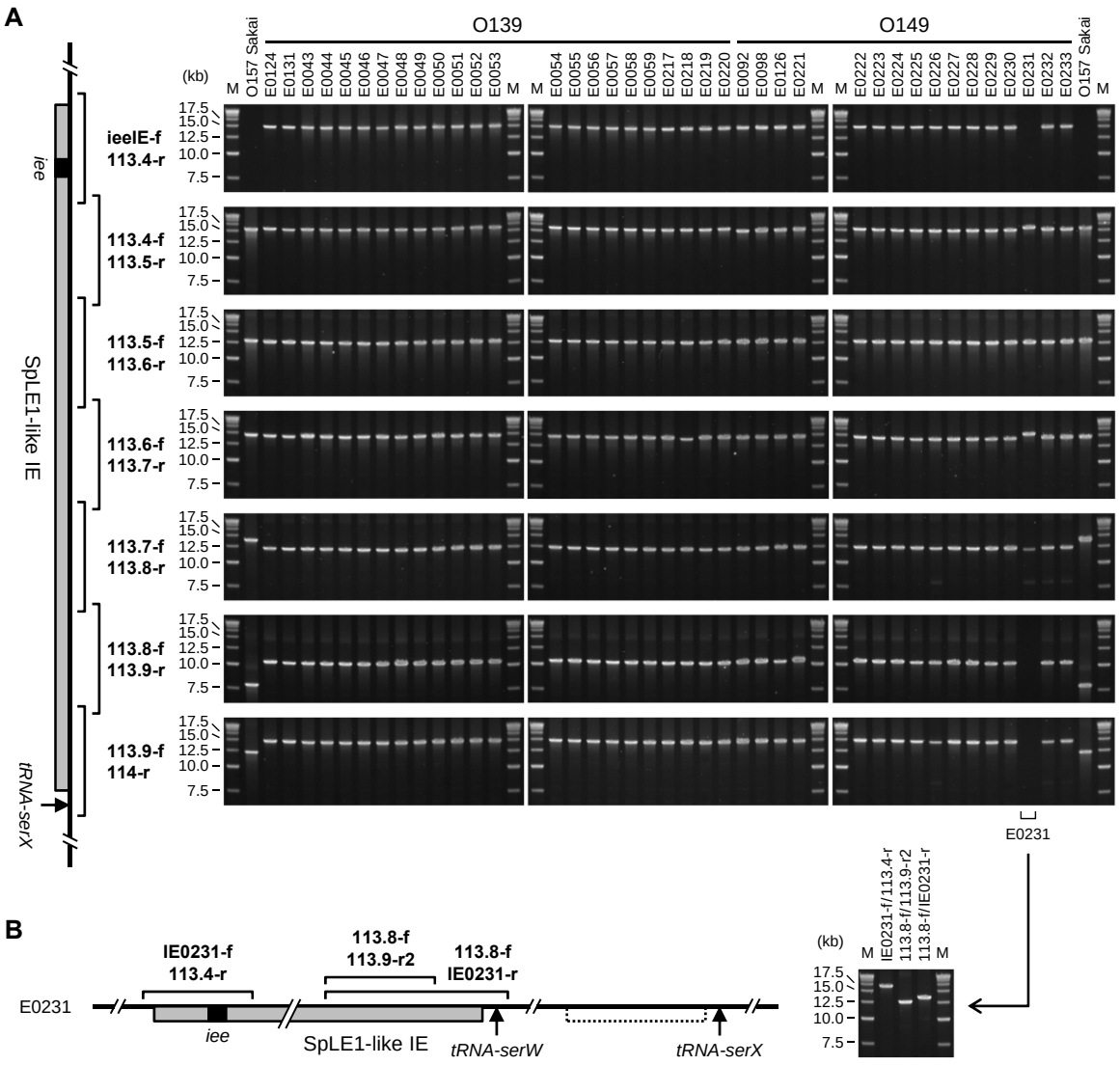


Figure S2. PCR scanning analysis of SpLE1-like IEs in the *iee*-positive O139 and O149 ETEC strains. (A) The results of the initial scanning are shown. O157 Sakai was analyzed as a control. The amplicons were analyzed on 0.4% agarose gels. (B) Three segments of strain E0231 that yielded no amplicon in the initial scanning were subjected to additional PCR analysis using newly designed primers. Note that the SpLE1-like IEs of all *iee*-positive O139 and O149 strains except for E0231 are integrated in the *serX* tRNA gene, as indicated by the dotted rectangles.

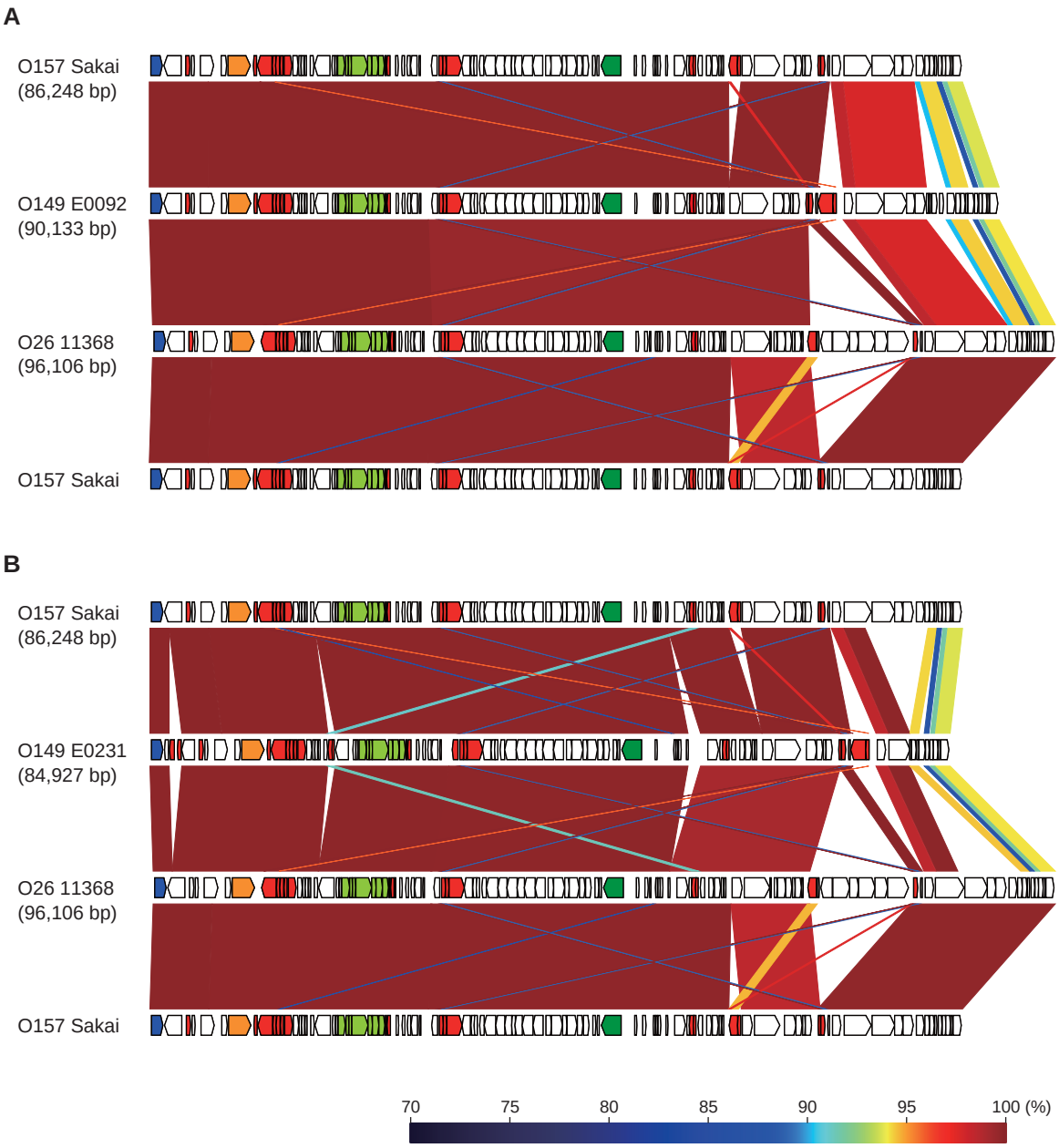


Figure S3. Genomic comparison of the SpLE1-like IEs of strain E0092 (A) and strain E0231 (B) with the elements of EHEC O157 and O26. The genes on each element and the sequence identities between the elements are represented as described in the legend for Fig. 4.