



FIG S1 (A) Operon into which ICESluvan has been integrated in *E. faecium* MM5-F9a compared to the corresponding region in *S. lutetiensis* 5-F9. Dark grey shading indicates regions of similarity between compared sequences. The tRNA (uracil-5)-methyltransferase *rumA* disrupted by insertion is coloured light grey. In *S. lutetiensis* 5-F9 the extended 5.7 kb region which is identical to parts of the *E. faecalis* G1-01247 *vanG* operon is shown. **(B)** Sequence comparison of the insert regions of ICESluvan in *E. faecium* MM5-F9a and *S. lutetiensis* 5-F9. Vertical lines indicate identical nucleotides. Left and right end sequences of ICESluvan are shown in uppercase. Amino acid residues of CDSs that have been disrupted upon the insertion of ICESluvan are marked (capital and bold) together with their respective codons. **(C)** Sequence comparison of the right end insert region of ICESluvan in *E. faecium* MM5-F9a and right end of the extended ICESluvan-like insertion element of *S. lutetiensis* 5-F9. The legend is the same as for (B).

Table S1. Control quality primer pairs used in the verification of ICESluvan sequence assembly in *E. faecium* MM5-F9a and to confirm different regions of the element in *E. faecalis* OG5-F9a and JH5-F9a, *S. lutetiensis* 5-F9 and NEM760 as well as *S. gallinarum* 4-C11 and 4-G10. + Indicates region confirmed with PCR by other primer combinations.

Primer	Sequence	ICESluvan position	Approximate PCR product size in kb						
			MM5-F9a	OG5-F9a	JH5-F9a	5-F9	NEM760	4-C11	4-G10
Q1.forw	gcactttcaagcatttgct	Orf2	2	2	2	2			
Q1.rev	tggaatggcgattttagctc	Orf5							
Q2.forw	gaagacagcccagtcatct	Orf5	3.1	+	+				
Q2.rev	gggttagacgatggcagaaa	Orf11/TraG							
Q3.forw	aatgctccaccttatggaaacg	Orf11/TraG	2.7	2.7	2.7	2.7	2.7	-	-
Q3.rev	ccttgaacggcatagaccat	Orf13/VirB6							
Q2.forw	gaagacagcccagtcatct	Orf5	+	5.8	5.8				
Q3.rev	ccttgaacggcatagaccat	Orf13/VirB6							
Q4.forw	tttgccctgttaccattc	Orf13/VirB6	2.6	2.6	2.6	2.6			
Q4.rev	acacggttccaaatctggtc	Orf15/VirB4							
Q5.forw	gaccagatttggAACCGTGT	Orf15/VirB4	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Q5.rev	atcccggttatctgtccat	Orf16/PG hydrolase							
Q6.forw	tttaggcttaggccaatggaa	Orf16/PG hydrolase	2.5	2.5	2.5	+ ^a	2.5	+	+
Q6.rev	cgatcatcacgtcgaaacac	Orf19/Tn1549 Orf13							
Q7.forw	gcttgatctggccgactat	Orf19/Tn1549 Orf13	2.5	2.5	2.5				
Q7.rev	gtccgacaggctcgatttcat	Orf21-22 intergenic							
Q8.forw	caagtggtaacgcaggatga	Orf21-22 intergenic	2.5	2.5	2.5	2.5			
Q8.rev	aaagatagccgtctgcgtgt	Orf26/Tn1549 Orf20							
Q9.forw	acacgcagacggctatcttt	Orf26/Tn1549 Orf20	2.5	2.5	2.5				
Q9.rev	cgttctccctccgacagttg	Orf27/Tn1549 Orf21							
Q10.forw	ccaaactgtcgaggaaagaac	Orf27/ Tn1549 Orf21	2.5	2.5	2.5				
Q10.rev	cgctgacggctatagtcg	Orf29/ Tn1549 Orf23							
Q11.forw	ccgtcagcggaaaaggaaatg	Orf29/ Tn1549 Orf23	2.3	+	+				
Q11.rev	gctttcgcatatgcctccag	Orf31/ TnpX							
Q12.forw	ctttgcgagggccagacett	Orf30/ Tn1549 Orf24	2.5	+	+				
Q12.rev	tttagtctgttgtggcgttg	Orf32							
Q13.forw	gcacggcagtagtgcagaga	Orf32	2.5	2.5	2.5	2.5			
Q13.rev	gcaagtggcttctctctgc	Orf35-36 intergenic							
Q14.forw	ggaagcgcaggtctatctga	Orf35	2.4	2.4	2.4	2.4			
Q14.rev	ggaaagagaggcaggtaacg	Orf37/DNA primase							
Q15.forw	acttccggcttgcgtgaaaa	Orf37/DNA primase	4.7	4.7	4.7				
Q15.rev	gtctgtccattttggcaag	Orf39 / Tn1549 Orf25							
Q16.forw	gctgttaggcgagttgtcc	Orf39 / Tn1549 Orf25	2.5	2.5	2.5				
Q16.rev	ttcaggctgtccacaatacg	Orf40/ Tn1549 Orf26							
Q17.forw	cgatttcaaagtccacgtca	Orf40/ Tn1549 Orf26	2.5	2.5	2.5				
Q17.rev	ctggaaaggcgaagctcaaag	Orf43/ Tn1549 Orf28							
Q18.forw	aaactttccggcttgcgtatct	Orf43/ Tn1549 Orf28	2.6	2.6	2.6				
Q18.rev	cgattacagccgttgtcaaa	Orf47-48 intergenic							
Q19.forw	ggcaagtggggatcagaat	Orf47-48 intergenic	2.5	+	+				
Q19.rev	cgcatttgcgtatgttgc	Orf50/ vanY _B							
Q20.forw	tatcattccaaatgcggatca	Orf50/ vanY _B	2.5	2.5	2.5	2.5		+ ^b	+ ^b
Q20.rev	cattcgcatcttaaaagc	Orf52/ vanH _B							
Q21.forw	gcttttaaggatgccgaatg	Orf52/ vanH _B	2.5	2.5	2.5	+ ^b	+	+ ^b	+ ^b

Q21.rev	tccaaatctgcgtgacaa	Orf55/ Tn1549 Orf7						
Q22.forw	cgttaacgaggccaaaagcta	Orf55/ Tn1549 Orf7	2.5	2.5	2.5			
Q22.rev	gttccattctgccttgc	Orf59/ Tn1549 int						
Q23.forw	attatgcacacgccacattc	Orf59/ Tn1549 int	2.5	2.5	+		2.5	+
Q23.rev	atccgcgtacagcttgtcg	Orf61/aggl. receptor						
Q24.forw	cgaacaagctgttagcgatt	Orf61/aggl. receptor	2.5	2.5	2.5			
Q24.rev	ggtccaagtcccatgaaaga	Orf61/aggl. receptor						
Q25.forw	ccgagggattaaagcagaca	Orf61/aggl. receptor	2.5	2.5	2.5		2.5	-
Q25.rev	ttccactcctcatcaaaatgc	Orf64/ helicase						
Q26.forw	gccaccattcctcggtatag	Orf64/ helicase	2.5	2.5	2.5			
Q26.rev	cgttgtgacactcggtctg	Orf64/ helicase						
Q27.forw	caccttcatccaaatagtcaca	Orf64/ helicase	2.5	+	+			
Q27.rev	gettgatttctctgagttgtc	Orf64/ helicase						
Q28.forw	ccgatgagtcgtgaaaaaca	Orf64/ helicase	2.5	2.5	2.5	2.5		
Q28.rev	aagttggcaaccaaataca	Orf65						
Q29.forw	ttgatttggcccaactt	Orf65	0.7	+	0.7			
Q29.rev	catccatgtattgaaacgaaa	Orf66-67 intergenic						
Q30.forw	tggttacaaaacaaactgcgaa	Orf66-67 intergenic	2	+	+	2		
Q30.rev	gggaccaaattgtatgggtta	Orf68						
Q31.forw	gccaaggcacactggaccat	Orf68	2.8	+	+			
Q31.rev	tgcctgtatgtgtcg	Orf70						
Q32.forw	ttggctatacgtatggctca	Orf70	2.4	+	2.4		2.4	-
Q32.rev	cgtttaattgtccgtgtctg	Orf74						
Q33.forw	cagaaccaggatcaattaaacg	Orf74	2.5	2.5	2.5		0.5 °	-
Q33.rev	gcaaagcttctggAACATT	Orf77						
Q34.forw	aaaatgttccaagaagcttgc	Orf77	2.5	2.5	2.5		-	-
Q34.rev	ggtatccgtttaatggtgaca	Orf79/MobC						
Q35.forw	ttgctgaatccaaatgtca	Orf79/MobC	2.4	2.4	2.4	2.5		
Q35.rev	tttgacactcttagcttcat	Orf80-81 intergenic						
Q36.forw	gagaagcttagagcaggtaaa	Orf80-81 intergenic	2.5	2.5	2.5			
Q36.rev	agccccagttatgtatgag	Orf82/ ABC permease						
Q37.forw	cagtttagccaaaaggaaatcca	Orf82/ ABC permease	2.7	2.7	2.7		2.7	
Q37.rev	ccgtctcttatacgttgAAC	Orf83/ histidine kinase						
Q38.forw	cgaataaaaatggcgaaaa	Orf83/ histidine kinase	3.6	+	+	+ d		-
Q38.rev	tctccgtcgatattactcca	Orf86/ resolvase						

The blank spaces in the columns indicate not examined

^a Tn5382/Tn1549 left end has been established previously by hybridization (20)

^b The *vanB2* cluster and link to Tn5382/Tn1549 has been established previously by other PCRs (20).

^c Sequence identical to 493 bp of ICESluvan orf77

^d Confirmed by end sequencing

1 **Table S2.** Features and predicted functions of ORFs in the ICESluvan element

ICESluvan	% G/C	% aa	Size	Product	Homolog	Species	NCBI reference
	content	identity	Aa				
1	52.4	85	13	C-terminal fragment of 23S rRNA (uracil-5)-methyltransferase RumA	SP70585_1067	<i>S. pneumoniae</i> 70585	CP000918
2	33.3	95	55	Hypothetical protein	SP70585_1068	<i>S. pneumoniae</i> 70585	CP000918
3	36.2	82	57	Hypothetical protein	SP70585_1069	<i>S. pneumoniae</i> 70585	CP000918
4	40.8	94	272	Replication initiator protein	ICESde3396_66	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
5	44.2	98	451	Cytosine-specific methyltransferase	ICESde3396_65	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
6	38.2	95	142	Hypothetical protein	ICESde3396_64	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
7	40.9	97	127	Hypothetical protein	ICESde3396_63	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
8	36.3	94	79	Hypothetical protein	ICESde3396_62	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
9	37.8	81	194	CAAX amino terminal protease self- immunity protein	ICESde3396_61	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
10	39.2	94	174	Hypothetical protein	ICESde3396_60	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
11	40.8	96	604	Conjugal transfer protein, TraG family coupling protein (pseudogene because of frame shift after codon 465)	ICESde3396_59	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
12	44.5	96	84	Putative membrane protein	ICESde3396_58	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
13	41.9	99	284	Putative plasmid conjugal transfer protein, TrbL/VirB6 family	ICESde3396_57	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
14	39.7	97	151	Hypothetical protein, PrgI family component	SP70585_1080	<i>S. pneumoniae</i> 70585	CP000918
15	40.8	99	776	Putative conjugal transfer protein, VirB4 ATPase	ICESde3396_56	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
16	43.1	99	933	<i>N</i> -acetylmuramoyl L-alanine amidase, peptidoglycan hydrolase	ICESde3396_55	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
17	32.6	94	281	Abortive infection protein AbiGII (pseudogene terminated by Tn1549 insertion after codon 251)	ICESde3396_54	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> NS3396	EU142041
18	52.3	100	399	Hypothetical protein	Tn1549 Orf 13	<i>E. faecalis</i> BM4382	AF192329
19	57.3	100	70	Hypothetical protein	Tn1549 Orf 14	<i>E. faecalis</i> BM4382	AF192329
20	53.6	99	160	Hypothetical protein	Tn1549 Orf 15	<i>E. faecalis</i> BM4382	AF192329
21	51.9	99	564	Putative conjugal transfer protein, TraG family coupling protein	Tn1549 Orf 16	<i>E. faecalis</i> BM4382	AF192329
22	57.4	100	82	Putative conjugal transfer protein	HMPREF0348_0460	<i>E. faecalis</i> (TX0104)	ACGL01000032
23	52.3	100	290	Putative conjugal transfer protein	Tn1549 Orf 17	<i>E. faecalis</i> BM4382	AF192329
24	54.1	100	180	Adenine-specific methyltransferase	Tn1549 Orf 18	<i>E. faecalis</i> BM4382	AF192329

25	52.0	100	140	Hypothetical protein	Tn1549 Orf 19	<i>E. faecalis</i> BM4382	AF192329
26	55.1	100	799	Putative conjugal transfer protein, VirB4 APTase	Tn1549 Orf 20	<i>E. faecalis</i> BM4382	AF192329
27	58.6	100	663	DNA-repair protein	Tn1549 Orf 21	<i>E. faecalis</i> BM4382	AF192329
28	52.4	100	83	Hypothetical protein	Tn1549 Orf 22	<i>E. faecalis</i> BM4382	AF192329
29	59.2	100	409	Putative bacteriocin	Tn1549 Orf 23	<i>E. faecalis</i> BM4382	AF192329
30	57.7	99	693	DNA topoisomerase III-like protein (pseudogene terminated by insertion of a 9-kb element after codon 474)	Tn1549 Orf 24	<i>E. faecalis</i> BM4382	AF192329
31	47.6	83	620	TnpX site-specific recombinase	FAEPRAA2165_00064	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
32	55.9	89	98	Hypothetical protein	FAEPRAA2165_00836	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
33	39.9	55	350	Transposase (pseudogene because of frame shift after codon 251), IS30 family	LJ0275	<i>Lactobacillus johnsonii</i> NCC 533	AE017198
34	31.7	40	162	Putative VanZ family protein	Pjdr2_3222	<i>Paenibacillus</i> sp. JDR-2	NC_012914
35	59.0	96	108	Hypothetical protein	FAEPRAA2165_00838	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
36	56.3	88	502	Putative mobilisation protein	FAEPRAA2165_00070	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
37	57.2	93	633	Putative DNA primase	FAEPRAA2165_00840	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
38	45.3	70	63	Hypothetical protein	FAEPRAA2165_00072	<i>F. prausnitzii</i> A2-165	NZ_ACOP02000000
39	58.1	100	1306	Hypothetical protein, LtrC family membrane associated enzyme involved in lipid metabolism	Tn1549 Orf 25	<i>E. faecalis</i> BM4382	AF192329
40	56.8	100	314	Hypothetical protein	Tn1549 Orf 26	<i>E. faecalis</i> BM4382	AF192329
41	49.5	100	129	Hypothetical protein	Tn1549 Orf 27	<i>E. faecalis</i> BM4382	AF192329
42	56.9	100	118	Hypothetical protein	HMPREF0348_0474	<i>E. faecalis</i> (TX0104)	ACGL01000032
43	52.3	100	442	Putative mobilisation protein, MOB _P family relaxase	Tn1549 Orf 28	<i>E. faecalis</i> BM4382	AF192329
44	53.0	100	109	Putative mobilisation protein, MobC	Tn1549 Orf 29	<i>E. faecalis</i> BM4382	AF192329
45	51.6	100	123	Hypothetical protein	Tn1549 Orf 30	<i>E. faecalis</i> BM4382	AF192329
46	52.2	100	61	Transposase	HMPREF0348_0478	<i>E. faecalis</i> (TX0104)	ACGL01000032
47	43.5	100	55	Hypothetical protein	HMPREF0348_0479	<i>E. faecalis</i> (TX0104)	ACGL01000032
48	46.5	100	220	Response regulator, VanRB	vanR _B	<i>E. faecalis</i> BM4382	AF192329
49	45.9	99	447	Sensor protein, histidine kinase VanSB	vanS _B	<i>E. faecalis</i> BM4382	AF192329
50	47.0	99	268	Carboxypeptidase VanYB	vanY _B	<i>E. faecalis</i> BM4382	AF192329
51	45.2	100	282	Vancomycin resistance protein VanW	vanW	<i>E. faecalis</i> BM4382	AF192329
52	50.6	100	323	Vancomycin resistance protein VanHB	vanH _B	<i>E. faecalis</i> BM4382	AF192329
53	49.8	100	342	Vancomycin B-type resistance protein VanB2	vanB	<i>E. faecalis</i> BM4382	AF192329
54	47.6	99	202	Vancomycin B-type resistance protein VanXB	vanX _B	<i>E. faecalis</i> BM4382	AF192329
55	51.2	100	258	Hypothetical protein	Tn1549 Orf7	<i>E. faecalis</i> BM4382	AF192329
56	55.6	100	77	Hypothetical protein	Tn1549 Orf8	<i>E. faecalis</i> BM4382	AF192329
57	58.0	100	80	Hypothetical protein	EFXG_01475	<i>E. faecium</i> C68	NZ_GG703717
58	43.3	100	66	Excisionase	Tn1549 xis	<i>E. faecalis</i> BM4382	AF192329
59	48.3	100	424	Integrase	Tn1549 int	<i>E. faecalis</i> BM4382	AF192329

60	35.4	89	196	Abortive infection protein AbiGI	ICESde3396_53	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
61	41.7	96	1631	Agglutinin receptor precursor	ICESde3396_52	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
62	41.7	92	63	Putative Ca ²⁺ binding protein	ICESde3396_50	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
63	38.6	96	183	Hypothetical protein	ICESde3396_49	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
64	41.1	95	2276	SNF2-related helicase (pseudogene because of frame shifts after codons 775 and 784)	ICESde3396_48	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
65	40.0	83	99	Hypothetical protein	ICESde3396_47	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
66	33.3	78	99	Hypothetical protein	ICESde3396_46	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
67	29.0	25	401	Putative uncharacterised phage related protein	StrS4_010100015457	<i>Streptomyces</i> sp. S4	NZ_CADY01000120
68	29.1	27	442	Putative uncharacterised phage related protein	StrS4_010100015462	<i>Streptomyces</i> sp. S4	NZ_CADY01000120
69	39.4	93	418	Transposase (pseudogene because of frame shift after codon 33), ISL3 family	SsuiDRAFT_2686	<i>S. suis</i> 89/1591	NZ_AAFA03000023
70	40.0	96	212	Hypothetical protein	ICESde3396_45	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
71	38.2	88	258	Putative DNA primase	ICESde3396_44	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
72	39.9	97	75	Hypothetical protein	ICESde3396_43	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
73	35.4	96	129	Hypothetical protein	ICESde3396_42	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
74	34.4	75	96	Hypothetical protein	ICESde3396_41	<i>S. dysgalactiae</i> subsp. equisimilis NS3396	EU142041
75	37.7	100	168	Antidote epsilon protein, zeta toxin regulator	SsuiDRAFT_3902	<i>S. suis</i> 89/1591	NZ_AAFA03000023
76	44.0	99	256	Zeta-toxin/ Signal recognition particle	SsuiDRAFT_3901	<i>S. suis</i> 89/1591	NZ_AAFA03000023
77	29.5	100	835	Hypothetical protein	SsuiDRAFT_3900	<i>S. suis</i> 89/1591	NZ_AAFA03000023
78	38.9	81	119	Hypothetical protein	SsuiDRAFT_3899	<i>S. suis</i> 89/1591	NZ_AAFA03000023
79	35.0	95	121	Putative mobilisation protein, MobC family	SsuiDRAFT_3898	<i>S. suis</i> 89/1591	NZ_AAFA03000023
80	34.7	92	621	Putative relaxase, MOB _P family	SsuiDRAFT_3897	<i>S. suis</i> 89/1591	NZ_AAFA03000023
81	34.3	82	249	ATPase component, ABC-type antimicrobial peptide transport system	smi_1460	<i>S. mitis</i> B6	FN568063
82	32.5	67	672	ABC transporter permease, ABC-type antimicrobial peptide transport system	smi_1459	<i>S. mitis</i> B6	FN568063
83	27.5	65	524	Histidine kinase	smi_1458	<i>S. mitis</i> B6	FN568063
84	30.5	74	198	Response regulator	smi_1457	<i>S. mitis</i> B6	FN568063
85	30.2	56	73	Transcriptional regulator, Cro/CI family	SsuiDRAFT_3888	<i>S. suis</i> 89/1591	NZ_AAFA03000023
86	33.6	98	579	Site-specific recombinase, resolvase family (lack 1 amino acid compared to SsuiDRAFT_2393)	SsuiDRAFT_2393	<i>S. suis</i> 89/1591	NZ_AAFA03000023