Supplemental Table 1. Primers used in this study.

Primer name	Sequence	Comments and expected product size
Closure of zero coverage regions		
EF0639 For	GCT TGT GAG AAG TCC AGC AA	538 bp
EF0639 Rev	CGC ACG TCG TTT ATT GCA AG	
ig EF1155-56 For	GCG AAT GTT ACT GAA GTT GAG	435 bp
ig EF1155-56 Rev	GCA CTT ACA TAC AGA CCG TC	
EF1797 For	CCA CTG TTA CTG GTG AAG TC	944 bp. Also used for serial passage cultures.
EF1797 Rev	GCA GTT CCT GTT GCT GCA AT	
EF2294 *vanB* For	CAC CTC TCT ATC GCC AAT GT	376 bp
EF2294 *vanB* Rev	GTC ATG GGA AAC GAG GAT GA	
ig EF2300-02 For	GTG GTT ATC AGA GCT TTC CG	419 bp
ig EF2300-02 Rev	CAT GGC AAT TAC AAG CAG GC	
EF2304 For	GTA GCA TGG GTA GCC TAC TT	532 bp
EF2304 Rev	CGT TAC CCA AGT CAG TCT GT	
EF2305 For	CGG ACA GCT TTA CTA ATG TG	223 bp
EF2305 Rev	GAG CTG CAA ATA CCA GAA CT	
EF2307 For	CCT CCA CCA TTT CTT TCT GG	378 bp
EF2307 Rev	CGC TAC ATG GAC GAA ATC AC	
EF2307 For 2	GCT TCC TCG CTG AAA TTC TG	227 bp
EF2307 Rev 2	GCA TGA GTA TTC GCT GTG GT	
EF2313 For	CGC CTA ATA CCT TAG ACA GC	273 bp
EF2313 Rev	GAC TAT CCT GAA AAC TAT GGC	
EF2314 For	GGT AGA ATA CGT TTT CAT CCG	349 bp
EF2314 Rev	GGA TAA CAA GCA GAA GCC CT	
EF2321 For	CAA TCG TCG ATA CCT CCT CA	327 bp
EF2321 Rev	CCC AAA GAC CTT GCC AAA GT	
Confirmation of polymorphisms		
EF0631 For A	GAG ACA GAA GTC GCT AAA GA	544 bp. Used to confirm DAP-A mutation.
EF0631 Rev A	GGT GCC GAT ATG ATC CGT TA	
EF0631 For BC	GCA ACA AAG TGA TTT ACG GG	754 bp. Used to confirm DAP-B/DAP-C mutation.
EF0631 Rev BC	GTT CAC GGT CAC TGT TAG GA	
brnQ For	GCG TTG TTA CAA ACG CCG TA	508 bp
brnQ Rev	CAG TGT TGT ATT GAT GGG GA	
EF2698 For	CTG GAG ATC AAA TGG ATG TC	292 bp. Also used for serial passage cultures.
EF2698 Rev	CTT CAG TAA GTC ATT GGT GG	
rpoN For	CTC AAC AGC TTC AAC AAT CC	458 bp. Also used for serial passage cultures.
rpoN Rev	CTC GTT CTG TTT GTA GCA TC	
EF1753 For	GAC ACT TAG ACT TTC TGG TG	334 bp. Also used for serial passage cultures.
EF1753 Rev	GTG ACA TTG ATG TGG ATG ATG	
rpsE For	CAA GCT TTA GCT GAA GCT GC	659 bp
rpsE Rev	CCT AAC GCT TTA ACT GTA GCA	
EF0716 For	GGT TAT GGT ATT GCT GGT TTC	505 bp. Also used for serial passage cultures.
EF0716 Rev	CAC ATG GAG GAA TCT TCG TC	

EF2470 For	GAA GCA GTC TTT CGA TAC GC	770 bp. Also used for serial passage cultures.
EF2470 Rev	CTG CGT TGA ACG CTA AAC TTA	
Profiling of EF0631 (*cls*) in *E. faecalis* clinical isolates (to obtain complete gene sequence)		
EF0631 For A	GAG ACA GAA GTC GCT AAA GA	Primer pair 1; 1028 bp. Also used for serial passage cultures and *E. faecalis* T2
EF0631 Rev BC	GTT CAC GGT CAC TGT TAG GA	
EF0631 close For	CGG AAA AGT AGG CTA CAT TG	Primer pair 2; 590 bp
EF0631 close Rev	CTT CGG ATA TCT TGA TTG GC	
EF0631 For 3prime	CCT AAC AGT GAC CGT GAA CA	Primer pair 3; 804 bp
EF0631 Rev 3prime	CTT GTA CAT GTA GCA TAT TCC T	
Profiling of EFTG_00614 (*cls*) in *E. faecium* clinical isolates (to obtain partial gene sequence)		
EFTG_00614 For	GCA CTT ATT GCT TTG GTG GC	1350 bp
EFTG_00614 Rev	CCT TGA CTG TTT CAG TTG TCA	
EFTG_00614 For 2 seq	CAC AAA GGG CAT TAC TTC GTT	Sequencing primers
EFTG_00614 Rev 2 seq	CGA AGC TGG AGC AAA GAA GA	
EF0631 cloning (for insertion into pAT28 multiple cloning site)		
EF0631 KpnI For	CGG GGT ACC GGG GTT GCT TAC TTT ACC GA	1733 bp for wt V583 and DAP-C; 1724 bp for DAP-A. Restriction enzyme cut sites are underlined.
EF0631 XbaI Rev	GCT CTA GAG ACG AAC TTC GGA TCA GAA G	
EF0631 clone check For	GCC TTT GTA ACG GAT CAT ATC	Used with EF0631 For A to confirm sequence of pAT28 inserts
pAT28 MCS Rev	CAC AGA TGC GTA AGG AGA AA	
EF0631 clone check Rev	CCC GTA AAT CAC TTT GTT GC	
M13 Forward (-20)	GTA AAA CGA CGG CCA G	277 bp. Product expected in putative pAT28 EF0631 clones.
EF0631 insert check Rev	GCC AAT CAA GAT ATC CGA AGT	

Supplemental Table 2. Resequencing assemblies.

Strain	Total reads[a]	Chr.[b]	Chr. coverage[c]	pTEF1[b]	pTEF1 coverage[c]	pTEF2[b]	pTEF2 coverage[c]	pTEF3[b]	pTEF3 coverage[c]	OLJ137[b]	OLJ131[b]	Non-assembled
Control	8,352,029	7,315,097	3-302 (90)	265,543	17-394 (159)	175,900	10-312 (121)	83,377	57-377 (185)	302,509	1,490	208,113
DAP-A	10,359,993	9,250,032	0-392 (109)	550,287	4-530 (315)	371,327	28-400 (245)	131,548	4-447 (278)	34,182	478	22,139
DAP-B	10,464,801	9,194,598	0-406 (109)	534,651	28-549 (306)	361,716	26-418 (238)	130,885	14-457 (277)	213,900	609	28,442
DAP-C	10,769,361	9,570,843	0-377 (113)	484,273	3-490 (277)	339,341	11-396 (224)	116,719	6-432 (247)	225,292	496	32,397

[a] Total single end reads generated by Illumina sequencing, after quality filtering.

[b] Number of reads aligned to reference sequence (chromosome, pTEF1, pTEF2, pTEF3, or Illumina adapters). Chr, chromosome.

[c] Range of fold coverage is shown; average fold coverage is in parentheses.

Supplemental Table 3. Zero coverage regions in Illumina resequencing assemblies.

Strain	ORF	Missing sequence relative to reference	Confirmation results
A	EF2294 *vanB*	CCGCCATG	Gap closed
A	EF2321	CGGCTCGTT	Gap closed
B	EF0639	CC	Gap closed
B	ig: EF1155-56[a]	GAGCCACAAGTCCAACCGACT	Gap closed
B	EF2294 *vanB*	AGC	Gap closed
B	ig: EF2300-02	CAGGCCGTC	Gap closed
B	EF2304	ATGTCGGCGCTGC	Gap closed
B	EF2305	CCGCGTCCCCCGCAGGCTG	Gap closed
B	EF2307	GT	Gap closed
B	EF2307	AAACTG	Gap closed
B	EF2313	AGGCAGGC	Gap closed
B	EF2314	GGCAG	Gap closed
C	EF1797	AAAAAGGTTTTTATTTTTTTGCGAACCGTATTTAC TGTAGATAATGGTTAAGACGCCTGATAAC	75 bp deletion
C	EF2294 *vanB*	G	Gap closed

[a] ig, intergenic region.

Supplemental Table 4. SNPs and DIPs in the control strain assembly.

Reference position	Variation type	Reference	Variations	Variation freq. (%)	Counts	Fold Cvg	Overlapping annotations	Amino acid change
132143	DIP	-	G	65.4	70	107		
179987	DIP	A	-	86.4	51	59	EF0183, hypothetical protein	Frameshift
191992	SNP	T	A	100.0	85	85	*rpsG*	
227635	DIP	A	-	81.9	95	116		
227646	DIP	C	-	77.9	102	131		
229297	DIP	C	-	90.3	93	103		
445423[a]	**Complex SNP**	**A**	**A/C**	**62.8/37.2**	**54/32**	**86**	**EF0479, site-specific recombinase, phage integrase family**	**Ser -> Arg**
611688	SNP	G	T	100.0	81	81	EF0660, MATE efflux family protein	Arg -> Ser
872056	SNP	C	A	100.0	94	94	EF0906, conserved hypothetical protein	
921232	SNP	A	G	100.0	61	61	EF0958, PTS system. IIABC components	
931162	SNP	G	T	100.0	68	68		
998111	SNP	C	T	100.0	93	93		
1170952	DIP	-	T	82.2	74	90	EF1205	
1303060	DIP	A	-	93.7	74	79		
1474120	SNP	C	A	100.0	52	52	EF1519, cation-transporting ATPase, E1-E2 family	Asn -> Lys
1534568	DIP	A	-	87.7	50	57	*tkt*	
1571355	SNP	C	T	98.6	72	73	*parC*	
1651141	SNP	T	C	100.0	46	46	EF1704, sensory box histidine kinase	
1651146	SNP	T	C	100.0	48	48	EF1704, sensory box histidine kinase	Leu -> Pro
1914897	DIP	-	G	86.0	74	86	*prmA*	Frameshift
1914901	DIP	G	-	84.9	62	73	*prmA*	Frameshift
1917084	SNP	T	A	100.0	73	73	EF1979, ATPase, AAA family	Val -> Asp
2059477	DIP	--	AA	71.1	59	83	EF2155	
2117617	DIP	-	C	85.7	66	77	EF2204	
2232992	DIP	T	-	80.0	20	25	EF2308, hypothetical protein	Frameshift
2234179	DIP	-	G	94.1	48	51		
2234474	SNP	C	G	100.0	39	39	*topB-2*	
2309182	SNP	C	A	100.0	115	115		
2323031	DIP	C	-	89.2	66	74	EF2399, acetyltransferase, GNAT family	Frameshift
2325873	DIP	-	G	100.0	42	42	EF2405, hypothetical protein	Frameshift
2452948	Complex DIP	-	-/C	50.0/50.0	3/3	6		
2452957	DIP	-	C	100.0	16	16		
2570780	SNP	T	C	98.7	76	77		
2578163	SNP	C	G	100.0	34	34		
2677895	DIP	A	-	94.9	93	98	EF2772	
3000492	DIP	-	C	94.1	64	68	*def-2*	
3015992	SNP	T	A	99.3	134	135		
3100859	DIP	G	-	100.0	65	65	*rplM*	
3125849	DIP	-	C	87.7	64	73	EF3251, hypothetical protein	Frameshift

[a] Complex SNP computationally detected only in the control V583 strain. Note that a similar A/C variation occurs in each of the DAP strains, but below the detection threshold (35%) used for SNP detection.