

Table S2. Plasmids used in this study.

	Plasmid identifier	Plasmid common name	Method used to derive from parent plasmid ^{a-b}	Parent plasmid	Antibiotic resistance ^c	Reference/source	Plasmid use in this study
A. Commercial vectors	n/a	pCR-Blunt	n/a	n/a	Kan	Invitrogen	Blunt-end cloning (see section E)
	n/a	pPCR-Script Amp (SK+)	n/a	n/a	Amp	Stratagene	Blunt-end cloning (see section F)
	n/a	pREP4	n/a	n/a	Kan	Qiagen	Encodes <i>lac</i> repressor to prevent expression from pQE-30Xa-derived plasmids (see Table S1 sections F-G)
	n/a	pQE-30Xa	n/a	n/a	Amp	Qiagen	IPTG-inducible expression of recombinant Ebp pilins with an N-terminal RGS-6×His tag in <i>E. coli</i> (section J)
B. Vectors used in the construction of pGCP213	n/a	pCR2.1	n/a	n/a	Amp, Kan	Invitrogen	Used in the construction of pGCP213 (see Text S1)
	n/a	pCR2.2	See Text S1	pCR2.1	Amp, Kan	This study	Used in the construction of pGCP213
	n/a	pCR2.3	See Text S1	pCR2.2	Amp, Kan	This study	Used in the construction of pGCP213
C. Published gram-positive cloning vectors	pSJH-460	pJRS233	n/a	n/a	Erm	(1)	Gram-positive temp-sensitive shuttle vector used for allelic replacement (see section G)
	pSJH-191	pABG5	n/a	n/a	Kan, Cam	(2)	Gram-positive expression vector
D. Gram-positive cloning vectors derived here	pSJH-329	pGCP123	See Text S1.	pABG5	Kan	This study	Gram-positive expression vector (see section I)
	pSJH-461	pGCP213	See Text S1.	pJRS233	Erm	This study	Gram-positive, temp.-sensitive shuttle vector used for allelic replacement in <i>E. faecalis</i> (see section H)
E. pCR-Blunt derivatives	pSJH-516	pCR-Blunt-EbpCA-X	Blunt-end ligation with EbpCA-X construct	pCR-Blunt	Kan	This study	Source of insert for pQE-30Xa-EbpCA-X (pSJH-550)
	pSJH-518	pCR-Blunt- Δ ebpC	Blunt-end ligation with Δ ebpC allele	pCR-Blunt	Kan	This study	Source of insert for pJRS233- Δ ebpC (pSJH-523)
	pSJH-520	pCR-Blunt- Δ ebpABC	Blunt-end ligation with Δ ebpABC allele	pCR-Blunt	Kan	This study	Source of insert for pJRS233- Δ ebpABC (pSJH-524)
F. pPCR-Script Amp (SK+) derivatives	pSJH-533	pPCR-Script- Δ ebpA	Blunt-end ligation with Δ ebpA allele	pPCR-Script	Amp	This study	Source of insert for pJRS233- Δ ebpA (pSJH-529)
	pSJH-534	pPCR-Script- Δ ebpB	Blunt-end ligation with Δ ebpB allele	pPCR-Script	Amp	This study	Source of insert for pJRS233- Δ ebpB (pSJH-530)
	pSJH-535	pPCR-Script- Δ ebpAB	Blunt-end ligation with Δ ebpAB allele	pPCR-Script	Amp	This study	Source of insert for pJRS233- Δ ebpAB (pSJH-531)
	pSJH-536	pPCR-Script- Δ ebpBC	Blunt-end ligation with Δ ebpBC allele	pPCR-Script	Amp	This study	Source of insert for pJRS233- Δ ebpBC (pSJH-532)
	pSJH-542	pPCR-Script-EbpA-X	Blunt-end ligation with EbpA-X construct	pPCR-Script	Amp	This study	Source of insert for pQE-30Xa-EbpA-X (pSJH-541)

	Plasmid identifier	Plasmid common name	Method used to derive from parent plasmid ^{a-b}	Parent plasmid	Antibiotic resistance ^c	Reference/source	Plasmid use in this study
G. pJRS233 derivatives	pSJH-189	pJRS233- Δ srtC	Ligation with Δ srtC allele (XbaI)	pJRS233	Erm	(3)	Creation of SrtC ⁻ and SrtC ⁻ SrtA ⁻ strains (SJH1424 and SJH2010)
	pSJH-279	pJRS233- Δ ebpABCsrtC	Ligation with Δ ebpABCsrtC allele (XbaI)	pJRS233	Erm	This study	Creation of EbpABC ⁻ SrtC ⁻ strain (SJH1995)
	pSJH-523	pJRS233- Δ ebpC	Ligation with pSJH-518 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpC ⁻ strain (SJH1424)
	pSJH-524	pJRS233- Δ ebpABC	Ligation with pSJH-520 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpABC ⁻ strain (SJH1422)
	pSJH-529	pJRS233- Δ ebpA	Ligation with pSJH-533 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpA ⁻ and EbpAC ⁻ strains (SJH1996 and SJH1999)
	pSJH-530	pJRS233- Δ ebpB	Ligation with pSJH-534 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpB ⁻ strain (SJH1997)
	pSJH-531	pJRS233- Δ ebpAB	Ligation with pSJH-535 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpAB ⁻ strain (SJH2000)
	pSJH-532	pJRS233- Δ ebpBC	Ligation with pSJH-536 insert (XbaI, Sall)	pJRS233	Erm	This study	Creation of EbpBC ⁻ strain (SJH1998)
H. pGCP213 derivative	pSJH-509	pGCP213-ebpA ^{AWAGA}	Ligation with ebpA ^{AWAGA} allele (XbaI, XhoI)	pGCP213	Erm	This study	Creation of EbpA ^{AWAGA} strain (SJH2001)
I. pGCP123 derivatives with the native ebpA promoter	pSJH-491	p-ebpABC	Ligation with ebpAp-ebpABC construct (XhoI, XmaI)	pGCP123	Kan	This study	Expression of EbpA, EbpB, and EbpC in <i>E. faecalis</i>
	pSJH-492	p-ebpAB	Ligation with ebpAp-ebpAB construct (XhoI, XmaI)	pGCP123	Kan	This study	Expression of EbpA and EbpB in <i>E. faecalis</i>
	pSJH-496	p-ebpABCsrtC	Ligation with ebpB (internal)-ebpC-srtC construct (StuI, XmaI)	p-ebpABC	Kan	This study	Expression of EbpA, EbpB, EbpC, and SrtC in <i>E. faecalis</i>
	pSJH-559	p-ebpA ^{AWAGA} BCsrtC	Ligation with ebpA ^{AWAGA} (internal) construct (SfoI, SbfI)	p-ebpABCsrtC	Kan	This study	Expression of EbpA ^{D315A, S317A, S319A} , EbpB, EbpC, and SrtC in <i>E. faecalis</i>
J. QE-30Xa derivatives	pSJH-541	pQE-30Xa-EbpA-X	Ligation with pSJH-542 insert (BamHI, Sall)	pQE-30Xa	Amp	This study	IPTG-inducible expression of N-term ^d :EbpA ⁶³⁴⁻¹¹¹⁷ in <i>E. coli</i>
	pSJH-547	pQE-30Xa-EbpB-X	Ligation with EbpB-X construct (SphI, PstI)	pQE-30Xa	Amp	This study	IPTG-inducible expression of N-term ^d :EbpB ²⁷⁻⁴⁸⁸ in <i>E. coli</i>
	pSJH-550	pQE-30Xa-EbpCA-X	Ligation with pSJH-516 insert (BamHI, SacI)	pQE-30Xa	Amp	This study	IPTG-inducible expression of N-term ^d :EbpC ³³⁻⁵⁹² in <i>E. coli</i>

^a For sections E-F, plasmids were derived by blunt-end cloning into the parent plasmid with the noted allele or construct (see Table S4).

^b For sections G-J, plasmids were derived by digestion of both the parent plasmid and the noted insert (see sections E-F) or allele/construct (see Table S4) with the restriction enzymes listed in parentheses and subsequent ligation.

^c Kan (kanamycin); Amp (ampicillin); Erm (erythromycin); Cam (chloramphenicol); concentrations used for growth of plasmid-carrying strains are listed in the main text Materials and Methods.

^d See Table S4 section D for the N-terminal amino acid sequence (N-term) added to the recombinant Ebp pilins by pQE-30Xa.

REFERENCES

1. **Perez-Casal J, Price JA, Maguin E, Scott JR.** 1993. *Mol. Microbiol.* **8**:809-819.
2. **Granok AB, Parsonage D, Ross RP, Caparon MG.** 2000. *J. Bacteriol.* **182**:1529-1540.
3. **Kline KA, et al.** 2009. *J. Bacteriol.* **191**:3237-3247.