

Table S2. Primer information

Primer	Plasmid	Sequence (5'-3') ^a	Source / reference
PpkA F-XhoI	pPpkA	5'-CCG <u>CTCGAGAGGGTAGCCGGA</u> CGTTTTC-3'	This study
PpkA R-XbaI		5'-GCT <u>CTAGACGTGTTCCAGAA</u> GAGTGTGC-3'	This study
PppA F-BamHI	pPppA	5'-CGG <u>GATCCTTATGATGAAGG</u> CAAGCACG-3'	This study
PppA R-XbaI		5'-GCT <u>CTAGAGGATAGGCAGGC</u> TCATCAAG-3'	This study
TssL F-XhoI	1. pTssL-His (TssL F-XhoI/TssL-His R-XbaI) 2. pTssL-Strep (TssL F-XhoI/TssL-Strep R-XbaI) 3. pTssL ^{T14A} -His (pJQ200KS- <i>tssL</i> ^{T14A} as template) (TssL F-XhoI/TssL-His R-XbaI) 4. pTssL ^{T14A} -Strep (pJQ200KS- <i>tssL</i> ^{T14A} as template) (TssL F-XhoI/TssL-Strep R-XbaI)	5'-CCG <u>CTCGAGTGCCATTGCCCT</u> GCATCTGG-3'	[1]
TssL-His R-XbaI		5'-GCT <u>CTAGATCAGTGGTGGTG</u> GTGGTGGTGTGGCTGGCCTCT CCC GGCT-3'	This study
TssL-Strep R-XbaI		5'-GCT <u>CTAGATCACTTTCGAAC</u> TGC GGGTGGCTCCATGGCTGGG CCTCTCCC GGCTG-3'	This study
TssL F-XhoI		5'-CCG <u>CTCGAGTGCCATTGCCCT</u> GCATCTGG-3'	[1]
TssL R-XbaI	pTssL ^{T14A} (pJQ200KS- <i>tssL</i> ^{T14A} as template)	5'-GCT <u>CTAGATCTCGACATAGG</u> AACGGATCG-3'	[1]
Fha F-NdeI		5'-GAAC <u>CATATGAACACACCGAA</u> CATCGGGAC-3'	This study
Fha ²⁶⁷ R-XhoI	1. pET-Fha7-267 ^{WT} -His 2. pET-Fha7-267 ^{R30AS46A} - His (pJQ200KS- <i>fha</i> ^{R30AS46A} as template)	5'-CCG <u>CTCGAGGCAATGGCAC</u> TTTCCCTTTC-3'	This study
Fha F-NdeI	pET-Fha7-309 ^{WT} -His	5'-GAAC <u>CATATGAACACACCGAA</u> CATCGGGAC-3'	This study
Fha ³⁰⁹ R-XhoI		5'-CCG <u>CTCGAGATGCATGAGGG</u> TTTCGAGC-3'	This study
PpkA 2F-BamHI	pJQ200KS- $\Delta t6$	5'-CGG <u>GATCCCTGTAGCGCCGG</u> CGTCAGTTG-3'	[2]
PpkA 2R-XmaI		5'-TCCCC <u>CCGGGGCCGTCAAGGA</u> GCGTGTACTTG-3'	[2]
Atu4352 2F-BamHI		5'-CGG <u>GATCCGAGTGACGACGA</u> TATCCAGC-3'	[2]
Atu4352 2R-XbaI		5'- <u>GCTCTAGACTCGATCTGAAAT</u> CACCGAG-3'	[2]
Fha 1F-XbaI	pJQ200KS- <i>fha</i> ^{ΔFHA}	5'-GCT <u>CTAGATGCCGAAGACAC</u> ACTTCTGC-3'	This study
Fha 2R-XmaI		5'-TCCCC <u>CCGGGGAGGAATGA</u> AATCCGGATCG-3'	This study
Fha ^{ΔFHA} -1		5'-GAC <u>CGCGCGCTTCGCCCTCA</u> AGGCCTCGCTCGAACAGCTCCACT G-3'	This study
Fha ^{ΔFHA} -2		5'-CAG <u>TGGAGCTTCGAGCGAGG</u> CCTTGAGGGCGAAAGCGCGCGT C-3'	This study

Fha 1F-XbaI	pJQ200KS- <i>fha</i> ^{R30A}	5'-GCTCTAGATGCCGAAGACAC ACTTCTGC-3'	This study
Fha 2R-XmaI		5'-TCCCCCCGGGGGAGGAATGA AATCCGGATCG-3'	This study
Fha ^{R30A} -1		5'-CAGTCGCAATCACGAGACGC GCCAATTGCGCGCGCCTC-3'	This study
Fha ^{R30A} -2		5'-CCGCCGCGCAATTGGCGCGT CTCGTGATTGCGACTGGCAG-3'	This study
Fha 1F-XbaI	1. pJQ200KS- <i>fha</i> ^{S46A} 2. pJQ200KS- <i>fha</i> ^{R30AS46A} (pJQ200KS- <i>fha</i> ^{R30A} as template)	5'-GCTCTAGATGCCGAAGACAC ACTTCTGC-3'	This study
Fha 2R-XmaI		5'-TCCCCCCGGGGGAGGAATGA AATCCGGATCG-3'	This study
Fha ^{S46A} -1		5'-GCGTCAATGCAGCTTGGCA ACGCGCCGCTCATTCATC-3'	This study
Fha ^{S46A} -2		5'-ACAATGAGCAGCGCTTGCC AAGCTGCATTGCACGCTGAG-3'	This study
TssL 1F-XbaI	pJQ200KS- <i>tssL</i> ^{T14A}	5'-GCTCTAGACGAAGACTGCAT CCAGCTTC-3'	[1]
TssL 2R-XmaI		5'-TCCCCCGGGAGTGTGCGATA AGGATCGCCTC-3'	[1]
TssL ^{T14A} -1		5'-CCTCGGTGATCTGACCACC GCCGCAAATCCTGCCAGG-3'	This study
TssL ^{T14A} -2		5'-CCTGGCAGGATTGCCGGCG GTGGTCGAGATCACCGAGG-3'	This study
TssL 1F-XbaI	pJQ200KS- <i>tssL</i> ^{T14D}	5'-GCTCTAGACGAAGACTGCAT CCAGCTTC-3'	[1]
TssL 2R-XmaI		5'-TCCCCCGGGAGTGTGCGATA AGGATCGCCTC-3'	[1]
TssL ^{T14D} -1		5'-CCTCGGTGATCTGACCACC TCCGCAAATCCTGCCAGG-3'	This study
TssL ^{T14D} -2		5'-CCTGGCAGGATTGCCGGAT GTGGTCGAGATCACCGAGG-3'	This study
TssL 1F-XbaI	pJQ200KS- <i>tssL</i> ^{T14E}	5'-GCTCTAGACGAAGACTGCAT CCAGCTTC-3'	[1]
TssL 2R-XmaI		5'-TCCCCCGGGAGTGTGCGATA AGGATCGCCTC-3'	[1]
TssL ^{T14E} -1		5'-CCTCGGTGATCTGACCACC TCCGCAAATCCTGCCAGG-3'	This study
TssL ^{T14E} -2		5'-CCTGGCAGGATTGCCGGAG GTGGTCGAGATCACCGAGG-3'	This study
PpkA 1F-XbaI	pJQ200KS- <i>ppkA</i> ^{D161ANI166A}	5'-GCTCTAGAGGAGATGATGGC ACAGCAGATC-3'	This study
PpkA 2R-XmaI		5'-TCCCCCGGGCCGTAGGA GCGTGTACTTG-3'	This study
PpkA ^{D161ANI166A} -1		5'-CATCCGCCAGCAGAAATAGCG GCTGGCGTGACGGCGCAATGC ACATATCC-3'	This study
PpkA ^{D161ANI166A} -2		5'-GGATATGTGCATTGCCGT CACGCCAGCCGCTATTCTGCTG GCGGATG-3'	This study

a: Restriction enzyme sites are underlined, and mutated sequences are indicated by bold type.

References

1. Ma LS, Lin JS, Lai EM (2009) An IcmF family protein, ImpL_M, is an integral inner membrane protein interacting with ImpK_L, and its walker a motif is required for type VI secretion system-mediated Hcp secretion in *Agrobacterium tumefaciens*. *J Bacteriol* 191: 4316-4329.
2. Lin JS, Ma LS, Lai EM (2013) Systematic Dissection of the *Agrobacterium* Type VI Secretion System Reveals Machinery and Secreted Components for Subcomplex Formation. *PLoS One* 8: e67647.