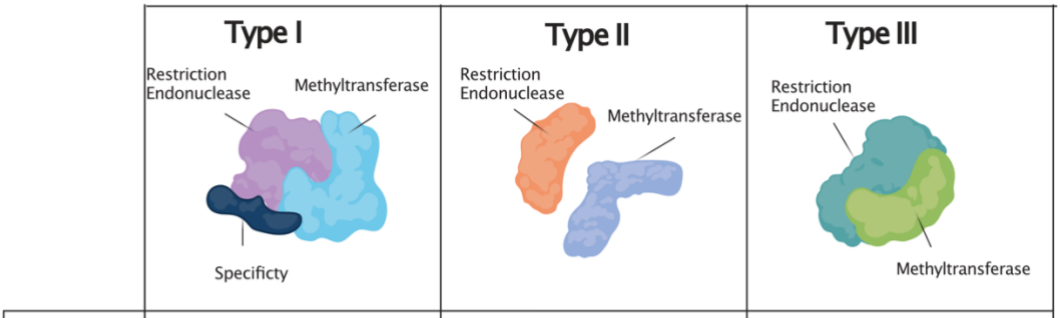
SUPPLEMENTAL MATERIAL

A



	Type I	Type II	Type III
Restriction Machinery	R_2M_2S	RE	R_2M_2
Methylation Machinery	M_2S	MT	M_2
Cleavage site	Variable	Fixed	Variable
Nucleobase	6mA	6mA, 5mC, 4mC	6mA, 4mC

B

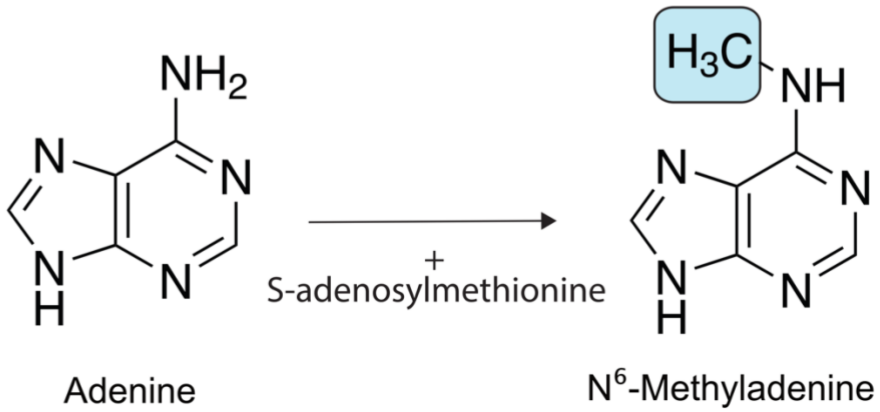


Figure S1. R-M systems and DNA methylation. (A) R-M systems are classified as Type I, Type II, and Type III according to their molecular structure, subunit composition, cleavage position, restriction site, and cofactor specification. **(B)** Nearly all methylation in *Fusobacterium* is predicted to be on adenine or adenosine residues within DNA and is added to nitrogen at the 6th position to create N6-Methyladenine (N^6-mA or ^{6}mA).

Table S1. *Fusobacterium nucleatum (Fn)* R-M system analysis in 25 strains.

***Fn* Strain**	**# Type I**	**# Type II Brex**	**# Type II**	**# Type III**	**# Type IV**	**Rebase Organism Number and link to strain analysis**
Fusobacterium nucleatum subsp. nucleatum ATCC 25586	0	0	3	1	0	28619 - Link
Fusobacterium nucleatum subsp. nucleatum ATCC 23726	1	1	2	0	0	28620 - Link
Fusobacterium nucleatum subsp. nucleatum ChDC F316	2	0	3	2	0	11922 - Link
Fusobacterium nucleatum subsp. nucleatum ChDC F317	2	0	3	2	0	25130 - Link

Fusobacterium nucleatum subsp. nucleatum KCOM 1250	0	1	4	2	0	17057 – Link
Fusobacterium nucleatum subsp. animalis 7_1	2	1	8	0	0	12464 - Link
Fusobacterium nucleatum subsp. animalis ChDC F324	1	1	5	0	0	11921 - Link
Fusobacterium nucleatum subsp. animalis ChDC F332	2	1	3	0	1	25138 - Link
Fusobacterium nucleatum subsp. animalis KCOM 1325	1	1	4	0	1	17056 - Link
Fusobacterium nucleatum subsp. animalis THCT5A4	1	2	4	0	3	50803 - Link
Fusobacterium nucleatum subsp. animalis THCT6B3	0	1	5	0	2	50804 - Link
Fusobacterium nucleatum subsp. animalis THCT7A2	1	1	2	1	2	50805 - Link
Fusobacterium nucleatum subsp. vincentii 3_1_27	2	1	4	0	0	12221 - Link
Fusobacterium nucleatum subsp. vincentii 3_1_36A2	2	1	1	1	0	11159 - Link
Fusobacterium nucleatum subsp. vincentii ATCC 49256	2	0	2	2	0	3870 - Link
Fusobacterium nucleatum subsp. vincentii ChDC F8	1	0	2	2	1	11920 - Link
Fusobacterium nucleatum subsp. vincentii KCOM 2931	3	0	2	0	2	26587 - Link
Fusobacterium nucleatum subsp. vincentii NCTC11326	1	2	2	0	0	43544 - Link
Fusobacterium nucleatum subsp. vincentii THCT14A3	3	1	2	0	2	50808 - Link
Fusobacterium nucleatum subsp. polymorphum 10953	2	0	4	0	0	5408 - Link
Fusobacterium nucleatum subsp. polymorphum ChDC F306	2	1	4	0	1	17423 - Link
Fusobacterium nucleatum subsp. polymorphum ChDC F319	2	1	3	0	1	17492 - Link
Fusobacterium nucleatum subsp. polymorphum KCOM 1275	2	0	3	1	0	25139 - Link
Fusobacterium nucleatum subsp. polymorphum THCT15E1	2	1	3	0	3	50806 - Link
Fusobacterium nucleatum subsp. polymorphum THCT7E2	1	1	4	2	2	50807 - Link

Table S2. *Fusobacterium nucleatum (Fn)* DNA methyltransferases (MTases) analyzed in this study from Figure 4

Name	*Fn* Strain	NCBI ID	MTase Type	REBASE Predicted Recognition and Methylation site	Link to REBASE classification of the MTase
M.Fnn23.I	*F. nucleatum* subsp. *nucleatum* ATCC 23726	AVQ22737.1	II	C**A**TG	M.Fnu23726SORF3380P

M.Fnn23.II	*F. nucleatum* subsp. *nucleatum* ATCC 23726	AVQ22751.1	II	GC**A**TC	M.Fnu23726SORF3460P
M.Fnn25.I	*F. nucleatum* subsp. *nucleatum* ATCC 25586	AVQ15832.1	II	CCNNNNNNNGG	M.Fnu25586ORF2505P
M.Fnn25.II	*F. nucleatum* subsp. *nucleatum* ATCC 25586	AVQ14558.1	II	C**A**TG	M.Fnu25586ORF3175P
M.Fnn25.III	*F. nucleatum* subsp. *nucleatum* ATCC 25586	AVQ14569.1	II	GC**A**TC	M.Fnu25586ORF3235P
M.Fnn25.IV	*F. nucleatum* subsp. *nucleatum* ATCC 25586	AVQ14879.1	III	GCATT	M1.Fnu25586ORF5130P
M.Fnn25.V	*F. nucleatum* subsp. *nucleatum* ATCC 25586	AVQ15904.1	III	GC**A**TT	M2.Fnu25586ORF5130P
M.Fna48.I	*F. nucleatum* subsp. *animalis 4_8*	AGM22521.1	II	G**A**NTC	M.Fsp48ORF1891P
M.Fna48.II	*F. nucleatum* subsp. *animalis 4_8*	AGM23250.1	II	CTNN**A**G	M.Fsp48ORF934P
M.Fna48.III	*F. nucleatum* subsp. *animalis 4_8*	AGM23714.1	III	GGC**A**S S=C/G	M.Fsp48ORF464P
M.Fna71.I	*F. nucleatum* subsp. *animalis 7_1*	EEO43724.1	II	G**A**NTC	M.Fnu71ORF2283P
M.Fna71.II	*F. nucleatum* subsp. *animalis 7_1*	EEO42819.1	II	G**A**TC	M.Fnu71ORF1378P
M.Fna71.III	*F. nucleatum* subsp. *animalis 7_1*	EEO42817.1	II	G**A**TC	M.Fnu71ORF1376P
M.Fna71.IV	*F. nucleatum* subsp. *animalis 7_1*	EEO42604.1	II	C**A**TG	M.Fnu71ORF1163P
M.Fna71.V	*F. nucleatum* subsp. *animalis 7_1*	EEO42568.1	II	G**A**NTC	M.Fnu71ORF1127P
M.Fna71.VI	*F. nucleatum* subsp. *animalis 7_1*	EEO42208.1	II	G**A**NTC	M.Fnu71ORF767P
M.Fna71.VII	*F. nucleatum* subsp. *animalis 7_1*	EEO43273.1	II	GC**A**TC	M.Fnu71ORF1832P
M.Fna71.VIII	*F. nucleatum* subsp. *animalis 7_1*	EEO43020.1	II	GC**A**TC	M.Fnu71ORF1579P
M.Fna71.IX	*F. nucleatum* subsp. *animalis 7_1*	EEO43011.1	II	No Prediction	M.Fnu71ORF1570P
M.Fnp10.I	*F. nucleatum* subsp. *polymorphum 10953*	EDK87839.1	II	G**A**TC	M.FnuPORF20P
M.Fnp10.II	*F. nucleatum* subsp. *polymorphum 10953*	EDK87614.1	II	GC**A**TC	M.FnuPORF2222P
M.Fnp10.III	*F. nucleatum* subsp. *polymorphum 10953*	EDK88996.1	II	CCWGG (C^5). W=A/T	M.FnuPORF1209P
M.Fnp10.IV	*F. nucleatum* subsp. *polymorphum 10953*	EDK89489.1	II	ATGC**A**T	M.FnuPORF1716P

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Table S3. Plasmids used in this study

Plasmid Name	Description	Source or Reference
pDJSVT7	Vector containing a *FLAG:galK* gene to make double crossover gene deletions in a Δ*galKT* background. (Cm^r Tm^r)	*Casasanta et al.*[1]
pDJSVT11	Chromosomal complementation vector for *F. nucleatum* 23726 and 25586. Incorporates a plasmid within the chromosomal *arsB* gene using homologous recombination. (Cm^r Tm^r)	*Casasanta et al.*[1]
pDJSVT13	Vector containing homologous regions +/- 1000 bp upstream and downstream of *galKT* for single crossover Integration in *F. nucleatum* 23726 and 25586 (Cmr, Tm^r)	*Casasanta et al.*[1]
pDJSVT21	pDJSVT13 with all of the CATG sites silently mutated. (Cmr, Tm^r)	This study

pDJSVT24	pET16b vector containing *m.fnn23.I* gene under a constitutive promoter. (Amp^r)	This study
pDJSVT25	pET16b vector containing *m.fnn23.II* gene under a constitutive promoter. (Amp^r)	This study
pDJSVT26	pET16b vector containing the *m.fnn23.I* and *m.fnn23.II* genes under independent constitutive promoters. (Amp^r)	This study
pDJSVT27	pET16b vector containing *m.fnn23.I* gene with a 6xHis tag. Under an IPTG inducible promoter for recombinant protein expression and purification. (Amp^r)	This study
pDJSVT28	pET16b vector containing *m.fnn23.II* gene with a 6xHis tag. Under an IPTG inducible promoter for recombinant protein expression and purification. (Amp^r)	This study
pDJSVT29	pET16b vector containing *m.fnn25.I* gene with a 6xHis tag. Under an IPTG inducible promoter for recombinant protein expression and purification. (Amp^r)	This study
pDJSVT30	pET16b vector containing *m.fnn25.IV* gene with a 6xHis tag. Under an IPTG inducible promoter for recombinant protein expression and purification. (Amp^r)	This study
pDJSVT31	pET16b vector containing *m.fnn25.V* gene with a 6xHis tag. Under an IPTG inducible promoter for recombinant protein expression and purification. (Amp^r)	This study
pTNVT2501	*fap2* gene deletion vector for *F. nucleatum* 25586 (Cm^r Tm^r)	This study
pTNVT2502	*fadA* gene deletion vector for *F. nucleatum* 25586 (Cm^r Tm^r)	This study
pDJSVT32	Chromosomal complementation vector for *fadA-FLAG F. nucleatum* 25586 Δ*galKT fadA*. Incorporates a plasmid within the chromosomal *arsB* gene expressing *fadA-FLAG* to complement strain TNVT2503 to make strain TNVT2508	This study
pET16b	IPTG inducible express vector. pDB322 origin of replication. (Amp^r)	EMD Millipore
pHS30	Fusobacterium multicopy, episomal pFN-1 based shuttle plasmid	*Kinder Haake et al.*[2]
pBAMD1-4	Standardized mini-Tn5 delivery plasmid for transposon mutagenesis. Streptomycin/spectinomycin resistant	*Martinez-Garcia et al.*[3]

Cm^r , Chloramphenicol resistance Tm^r , Thiamphenicol resistance. Amp^r, Ampicillin resistance

Table S4. Bacterial strains used in this study

Strain	Bacterial Species	Genotype and Characteristics	Source or Reference
TOP10	*E. coli*	*mcrA , Δ(mrr-hsdRMS-mcrBC), Phi80(del)M15, Δ lacX74 , deoR , recA1 , araD139 , Δ(ara-leu)7697, galU , galK , rpsL (SmR), endA1 , nupG*	Invitrogen
ArcticExpress (DE3) RIL	*E. coli*	B F– *ompT endA Hte [cpn10 cpn60 Gentr] hsdS(r8–m8) dcm+ Tetr galλ (DE3) [argU ileY leuW Strr]*	Agilent
LOBSTR-BL21(DE3)-RIL	*E. coli*	F- *ompT hsdSB (rB- mB-) dcm gal (DE3)*	*Anderson et al.*[4]
ER2796	*E. coli*	F- *fhuA2::IS2*, *glnX44*(AS), *λ*$^-$, *e14-*, *trp-31*, *dcm-6*, *yedZ3069::Tn10*, *hisG1*, *argG6*, *rpsL104*, *Δdam-16::KanR*, *xyl-7*, *mtlA2*, *metB1*, *Δ(mcrC-mrr)114:IS10* Methylation negative	*Anton et al.*[5]
F. nucleatum subsp. *nucleatum* ATCC 23726	*F. nucleatum*	Wild Type	ATCC
F. nucleatum subsp. *nucleatum* ATCC 25586	*F. nucleatum*	Wild Type	ATCC
F. nucleatum subsp. *animalis* 4_8	*F. nucleatum*	Wild Type	*Manson McGuire et al.*[6]
F. nucleatum subsp. *animalis 7_1*	*F. nucleatum*	Wild Type	*Manson McGuire et al.*[6]
F. nucleatum subsp. *polymorphum* 10953	*F. nucleatum*	Wild Type	*Manson McGuire et al.*[6]
TNVT2501	*F. nucleatum*	*F. nucleatum* 25586 *ΔgalKT* In-frame deletion of *galK* and *galT* genes (Base strain for all target in-frame gene deletions in *F. nucleatum* 25586)	This study
TNVT2502	*F. nucleatum*	*F. nucleatum* 25586 *ΔgalKT fap2* In-frame deletion of *fap2* in the TNVT2501 background	This study

TNVT2503	*F. nucleatum*	*F. nucleatum* 25586 *ΔgalKT fadA* In-frame deletion of *fadA* in the TNVT2501 background	This study
TNVT2508	F. nucleatum	*F. nucleatum* 25586 *ΔgalKT ΔfadA arsB::FLAG-fadA* Complementation strain of *ΔfadA*. (Cmr, Tm^r)	This study

19 Cm^r , Chloramphenicol resistance Tm^r , Thiamphenicol resistance.

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21 **Table S5. DNA oligonucleotides (primers) used in this study**

Primer Name	Sequence (5' to 3')	Description
prDJSVT1220	GTGGAGGAGCAGGCTATATTGGTAGCGATGTTGTTAAATATTTGTTAG	Forward Quikchange primer to remove CATG site 1 from pDJSVT13 to make pDJSVT21
prDJSVT1221	CTAACAAATATTTAACAACATCGCTACCAATATAGCCTGCTCCTCCAC	Reverse Quikchange primer to remove CATG site 1 from pDJSVT13 to make pDJSVT21
prDJSVT1222	CTTGCATAAGAGACTATATTGATGTAATGGACTTAGCAGATGCTCATTATC	Forward Quikchange primer to remove CATG site 2 from pDJSVT13 to make pDJSVT21
prDJSVT1223	GATAATGAGCATCTGCTAAGTCCATTACATCAATATAGTCTCTTATGCAAG	Reverse Quikchange primer to remove CATG site 2 from pDJSVT13 to make pDJSVT21
prDJSVT1224	GTGTACCTTGTACATACAGTATGACCGTTAAAGTGGATATCAC	Forward Quikchange primer to remove CATG site 3 from pDJSVT13 to make pDJSVT21
prDJSVT1225	GTGATATCCACTTTAACGGTCATACTGTATGTACAAGGTACAC	Reverse Quikchange primer to remove CATG site 3 from pDJSVT13 to make pDJSVT21
prDJSVT1226	GAAGATCCTTTTTGATAATCTGATGACCAAAATCCCTTAACGTGAG	Forward Quikchange primer to remove CATG site 4 from pDJSVT13 to make pDJSVT21
prDJSVT1227	CTCACGTTAAGGGATTTTGGTCATCAGATTATCAAAAAGGATCTTC	Reverse Quikchange primer to remove CATG site 4 from pDJSVT13 to make pDJSVT21
prDJSVT1068	CTGAGATCTAGATTTACAGCTAGCTCAGTCC	Forward primer to clone *Fnn* 23726 *m.fnn23.I* gene from synthetic codon optimized DNA into pET16b under a constitutive promoter. Has XbaI. To make pDJSVT24 and pDJSVT26
prDJSVT1069	GACTCTCCATGGAAATAATAAAAAAGCCGGATTAATAATCTG	Reverse primer to clone *Fnn* 23726 *m.fnn23.I* gene from synthetic codon optimized DNA into pET16b under a constitutive promoter. Has NcoI. To make pDJSVT24 and pDJSVT26
prDJSVT1070	CTGAGACCATGGTTTACAGCTAGCTCAGTCC	Forward primer to clone *Fnn* 23726 *m.fnn23.II* gene from synthetic codon optimized DNA into pET16b under a constitutive promoter. Has NcoI. To make pDJSVT25 and pDJSVT26
prDJSVT1071	GACTCTCATATGAAATAATAAAAAAGCCGGATTAATAATCTG	Reverse primer to clone *Fnn* 23726 *m.fnn23.II* gene from synthetic codon optimized DNA into pET16b under a constitutive promoter. Has NdeI. To make pDJSVT25 and pDJSVT26
prDJSVT1076	GCACTACCCATGGGAAACTATATCGGCAGCAAATTAAGtTTAAAAAG	Forward primer to clone *Fnn* 23726 *m.fnn23.I* gene from synthetic codon optimized DNA into pET16b under IPTG promoter. Has NcoI. To make pDJSVT27
prDJSVT1077	GCTAGTCTCGAGTTAgtgatggtgatggtgatgTTTCTTAATCAGGCAATGCAGATATTC	Reverse primer to clone *Fnn* 23726 *m.fnn23.I* gene from synthetic codon optimized DNA into pET16b under IPTG promoter. Has 6xHis and XhoI. To make pDJSVT27
prDJSVT1078	GCACTACCCATGGGAGAAAATCTGAAATTGTTCCTCGATG	Forward primer to clone *Fnn* 23726 *m.fnn23.II* gene from synthetic codon optimized DNA into pET16b under IPTG promoter. Has NcoI. To make pDJSVT28
prDJSVT1079	GCTAGTCTCGAGTTAgtgatggtgatggtgatgCATTTCTTCGGTGTTGTAGTTGGTC	Reverse primer to clone *Fnn* 23726 *m.fnn23.II* gene from synthetic codon optimized DNA into pET16b under IPTG promoter. Has 6xHis and XhoI. To make pDJSVT28
prAUT104	GATCCCCATGGGAAGTTATAAAGAAAAGATACTAAGTTTATTAAATGAAAATTTG	Forward primer to clone *m.fnn25.I* from genomic DNA into pET16b. Has NcoI site. To make pDJSVT29.
prAUT104	GACTCCCTCGAGTTAATGATGATGATGATGATGATTTTTAATAAAGTCGTTTATTCTTGAATATGC	Reverse primer to clone *m.fnn25.I* from genomic DNA into pET16b. Has 6XHis and XhoI site. To make pDJSVT29
prAUT91	GATCCCCATGGGAATATATATTGATCCTCCATATAATACAGGAAAAG	Forward primer to clone *m.fnn25.IV* from genomic DNA into pET16b. Has NcoI site. To make pDJSVT30.

prAUT92	GACTCCCTCGAGTTAATGATGATGATGATGATGTATACTTCTAATTTCACTTATTCCAACTTG	Reverse primer to clone *m.fnn25.VI* from genomic DNA into pET16b. Has 6XHis and XhoI site. To make pDJSVT30
prAUT93	GACTCCCCATGGGAGAAAAACTAAATGGAACAAGCATGG	Forward primer to clone *m.fnn25.V* from genomic DNA into pET16b. Has NcoI site. To make pDJSVT31.
prAUT94	GGATCCCTCGAGTTAATGATGATGATGATGATGTATACTCCTAATTTCATTTATTCCAACTTG	Reverse primer to clone *m.fnn25.V* from genomic DNA into pET16b. Has 6XHis and XhoI site. To make pDJSVT31
prTN17	GATCGCGGTACCGTAGTTATAGCTATATTTATTCCATTTGTAGGAGC	Forward primer -750 bp upstream of *fap2* in *Fnn* 25586. Has a KpnI site. Makes construct pTNVT2501
prTN18	CTATATACACTAGGGTTAGTGCTAATTTAATTATAAAGTGGTGCACTTGGTGCTG	Reverse primer -1 bp upstream of *fap2* in *Fnn* 25586. Overlaps with prTN19 for OLE-PCR. Makes construct pTNVT2501
prTN19	CTTTATAATTAAATTAGCACTAACCCTAGTGTATATAG	Forward primer +1 bp downstream of *fap2* in *Fnn* 25586. Overlaps with prTN18 for OLE-PCR. Makes construct pTNVT2501
prTN20	GATCGCACGCGTCTAAAAAATTTGTATTTTCTAGTAGACCTAAAAATTC	Reverse primer +750 bp downstream of *fap2* in *Fnn* 25586. Has an MluI site. Makes construct pTNVT2501
prTN21	GAAAATTCAATTTTGGAATTACTGGAACTTTATTTATTG	Forward gene deletion confirmation primer -1000 bp upstream of *fap2* in *Fnn* 25586
prTN22	CTTCTTCAAAATAATGAACATACATTTGCATTTG	Reverse gene deletion confirmation primer +1000 bp downstream of *fap2* in *Fnn* 25586
prTN23	GATAAAGATGCTGGAAAAAATACTATTCCAG	Forward sequencing primer -250 bp upstream of *fap2* *Fnn* 25586
prTN24	CTTTATTTCTTGCTTGTTCTAAAATACTTTTAATTTC	Reverse sequencing primer +250 bp upstream of *fap2* *Fnn* 25586
prTN49	GATCGCGGTACCCTATTAAAAAAAGCAAAAGAAGCTCAATATACAAATTATG	Forward primer -750 bp upstream of *fadA* in *Fnn* 25586. Has a KpnI site. Makes construct pTNVT2502
prTN50	GGTTTTATTTCATGCTAGCATTTTTTCAAAATTTATTTTGTTACCTCCCAAATTAAATTATAATAAATTATTTCTTATATTGAC	Reverse primer -1 bp upstream of *fadA* in *Fnn* 25586. Overlaps with prTN51 for OLE-PCR. Makes construct pTNVT2502
prTN51	TAAATTTTGAAAAAATGCTAGCATGAAATAAAACC	Forward primer +1 bp downstream of *fadA* in *Fnn* 25586. Overlaps with prTN50 for OLE-PCR. Makes construct pTNVT2502
prTN52	GATCGCACGCGTGCATAATCAAGTCCTGTATTGGCATTATTTAAG	Reverse primer +750 bp downstream of *fadA* in *Fnn* 25586. Has an MluI site. Makes construct pTNVT2502
prTN53	GTCAAAAATAAAAATATTATAAAAGTAGAGAGAAACTCTTG	Forward gene deletion confirmation primer -900 bp upstream of *fadA* in *Fnn* 25586
prTN54	CTTTCAAAGACAACATTGATGAATTAAAATTTGC	Reverse gene deletion confirmation primer +900 bp downstream of *fadA* in *Fnn* 25586
prTN55	CTTGCAGATGTTAAAAGAAATATATTTGGGC	Forward sequencing primer -250 bp upstream of *fadA* in *Fnn* 25586
prTN56	GCTACAACTGTAATTACAACTGCATAAAAC	Reverse sequencing primer +250 bp upstream of *fadA* in *Fnn* 25586
prTN89	GTATTTGTACCATCACTTAAACTGGTATGTG	Reverse Internal primer in *fap2* used to confirm gene deletion in *Fnn* 25586. Used with prTNVT21
prDJSVT847	GTAGGTGAATTACAAGCATTAGATGCTG	Forward primer of central region in *fadA* to confirm complementation in *Fnn* 25586
prDJSVT848	CCATTTCAGATTCTAATTTCTTTAAAGCATC	Reverse primer of central region in *fadA* to confirm complementation in *Fnn* 25586

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