

## Supplemental information

### **Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from *Francisella novicida***

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## Supplemental Figure Legends

### Figure S1. Heteroduplex mobility assay of *crNtSTF1*-1~3 loci.

PCR products were amplified from *crNtSTF1*-1 (top two panels), *crNtSTF1*-2 (middle two panels) and *crNtSTF1*-3 (bottom two panels) loci. Those products were subjected to heteroduplex mobility assay using MultiNA. (S) and (T) indicate PCR products amplified from the loci including each target sequence on *N. sylvestris* and *N. tomentosiformis* genomes, respectively.

### Figure S2. *dl* mutant phenotype at T0 generation.

Both plant were transgenic rice possessing *FnCpf1(Os)* with *crOsDL-2*. *dl/dl* indicates plant having biallelic mutation on *crOsDL-2* locus (Left side). *DL/DL* indicates plant without mutation on *crOsDL-2* locus (Right side).

### Figure S3. CAPS analysis of *crOsDL-1* locus in T1 generation of line #18.

-: Non-digested PCR products, +: *Pst* I-digested PCR products. Arrow head indicated the position of undigested PCR products. An undigested band indicates mutation in the target locus.

### Figure S4. CAPS analysis of *OsNCED* or *OsAO* gene families to survey off-target mutation.

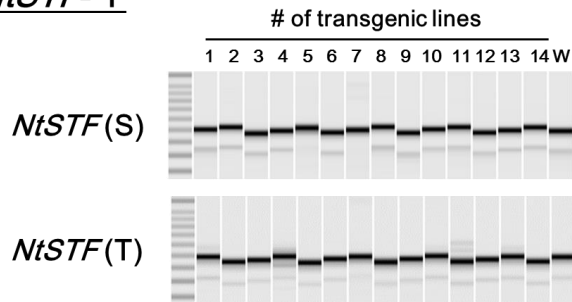
CAPS analysis of *crOsNCED1-1* and *crOsAO1-1* loci. -: Non-digested PCR products, + : *Sac* I or *Nde* I-digested PCR products. Arrow head indicated the position of undigested PCR products. An undigested band indicates mutation in the target loci.

**Table S1. List of primers used in this study**

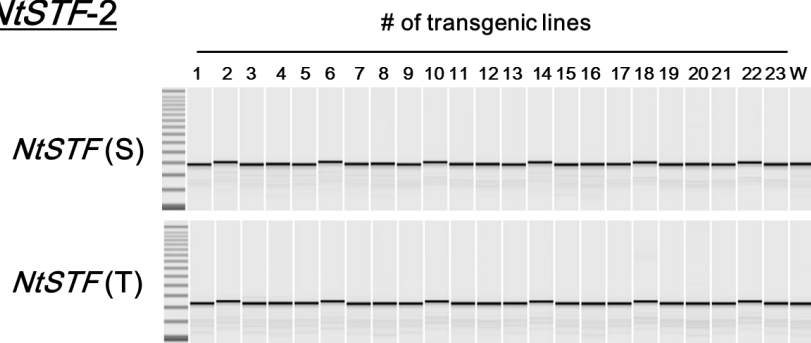
Plant species	Primer	Sequence	Application	
<i>Nicotiana tabacum</i>	NIPDS-1(S) F	ATAGCTGCTGCTGAGATTTGTAAAG	crNIPDS-1 HMA	
	NIPDS-1(S) R	GCCATACTAAAGAACAACGAAATGCTTACG	crNIPDS-1 HMA	
	NIPDS-1(T) F	AAATAGCTGCTGATGAGTTTTGCAAG	crNIPDS-1 HMA	
	NIPDS-1(T) R	GGCCATACTAAAGAACAATGAAATGCTTACA	crNIPDS-1 HMA	
	NIPDS-2(S&T) F	GGATTCTAACCTGAAGAAATCTGTTCAAAGC	crNIPDS-2 HMA	
	NIPDS-2(S&T) R	TGGATGAGAAAGCAAGTGCCTG	crNIPDS-2 HMA	
	NISTF-1(S) F	AGCAGGAGGAGTGGCAGAAT	crNISTF-1(S) HMA	
	NISTF-1(S) R	CTACTGCATATTATTGGAGAAGTAGTTCAGAT	crNISTF-1(S) HMA	
	NISTF-1(T) F	GCAGCAGGAGTGGCAGAATG	crNISTF-1(T) HMA	
	NISTF-1(T) R	GACTACTACTGCAAAATATTGGAGTAGTAATAG	crNISTF-1(T) HMA	
	NISTF-2(S) F	CTACTGCAGCAATAGTAATACTATATCTGGA	crNISTF-2(S) HMA	
	NISTF-2(S) R	AACAATGTTTTCCATATTTTCGTGTTTACG	crNISTF-2(S) HMA	
	NISTF-2(T) F	TCATCTACTGCATCAATCAGTAATACTATTACTAC	crNISTF-2(T) HMA	
	NISTF-2(T) R	AAGATTGTTTTCGATATTTTCGTGTTTGT	crNISTF-2(T) HMA	
	NISTF-3(S) F	GCAGCAGCAATGATAACAACGACGATA	crNISTF-3(S) HMA	
	NISTF-3(S) R	CATACACATGATCAAAATGCATTTACG	crNISTF-3(S) HMA	
	NISTF-3(T) F	GCAGCAGCAACGACGATAATAATC	crNISTF-3(T) HMA	
	NISTF-3(T) R	CATAGACATGGTCCAAATGCATTTTAAAG	crNISTF-3(T) HMA	
	NISTF-4(S) F	GGCACCATGATCCATCCTATCAA	crNISTF-4(S) CAPS	
	NISTF-4(S) R	CATACACATGATCAAAATGCATTTACG	crNISTF-4(S) CAPS	
	NISTF-4(T) F	GGCACCATGATCCATCCTATCAC	crNISTF-4(T) CAPS	
	NISTF-4(T) R	CATAGACATGGTCCAAATGCATTTTAAAG	crNISTF-4(T) CAPS	
		Tar-Cpf1-crRNA_NIPDS-1F	agatTCATCCAGTCTTAACACTTAAAC	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_NIPDS-1R	aaaaGTTTAAAGTGTAAAGGACTGGATGA	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_NIPDS-2F	agatACATGGCAATGAACACCTCATCTG	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_NIPDS-2R	aaaaCAGATGAGGTTCATTGCCATGT	cloning of crRNA into <i>Bbs</i> I site
	Tar-Cpf1-crRNA_NISTF-1F	agatCTAGCTGATCAAAGGAATGCCACG	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-1R	aaaaCGTGGCATTCTTTGATCAGCTAG	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-2F	agatGCTCCATTGTCGTCTTGGTGTG	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-2R	aaaaCAACACCAAGAACGACAATGGAGC	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-3F	agatTAAGTGGAAAGAACTCAAAAAACT	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-3R	aaaaAGTTTTTTGAGTTCTTCCACTTA	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-4F	agatAGAGAAGGATGAAGTAGAGATATC	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NISTF-4R	aaaaGATATCTCTACTTCATCCTTCTCT	cloning of crRNA into <i>Bbs</i> I site	
	AtU6-26 F	ATCTGTTCATAGTTTGTCCAGG	crRNA sequence check	
<i>Oryza sativa</i>	OsDL-1F	cagtgatcatgtccatctttcgcttccatt	crOsDL-1 CAPS	
	OsDL-1R	atgggcaagagagaaatcttttgcattcca	crOsDL-1 CAPS	
	OsDL-2F	tgcaaaagatttctctcttggccatctgtg	crOsDL-2 CAPS	
	OsDL-2R	ttctctcaCTCATGAAGCGGTGTGAAGCAG	crOsDL-2 CAPS	
	OsALS-1F	AATTATGCCGTGGATAAGGCTGACCTGTTG	crOsALS-1,2 CAPS	
	OsALS-1R	acccaataagatcgaccgagagaggggaaa	crOsALS-1,2 CAPS	
	OsNCED1-1F	GTCGGGAAGAGTGCATTTGGCTCCCGAA	crOsNCED1-1 CAPS	
	OsNCED1-1R	CTTCTTGATCACGCTAGCTGAGCGCGTG	crOsNCED1-1 CAPS	
	OsNCED2-1F	CGCGGAGGAGGTGGCAAGAAGAGGATG	crOsNCED1-1 CAPS off-target	
	OsNCED2-1R	GGCTGCTCAAGCTCGATCTCGACGTCGGGT	crOsNCED1-1 CAPS off-target	
	OsNCED3-1F	GCAACCGTCCCAACCAATGGCAAGTCAAG	crOsNCED1-1 CAPS off-target	
	OsNCED3-1R	CCGTGGCGGTGAAGTAGAAGTACTTGAGG	crOsNCED1-1 CAPS off-target	
	OsAAO1-1F	ACATCATCATGGCCAGAGGTTGTCTTCT	crOsAAO1-1 CAPS	
	OsAAO1-1R	AGAGAATATGTACCAGAAGCTTGGAGCTCA	crOsAAO1-1 CAPS	
	OsAAO2-1F	ACATCATCATGGCCAGAGGCTGTCTTCC	crOsAAO1-1 CAPS	
	OsAAO2-1R	TCTAAGATGCTCTAACATGGGTAGCACGAA	crOsAAO1-1 CAPS	
	OsAAO3+4-1F	GGCACACAATACCCTTTTCGCTCAGACAT	crOsAAO1-1 CAPS off-target	
	OsAAO3-1R	TATCCTTGCCAGAAACAAGTGAAGGATCTTCTTT	crOsAAO1-1 CAPS off-target	
	OsAAO4-1R	ACCACAACCCGAGAAGTGTCCAGCAAAC	crOsAAO1-1 CAPS off-target	
	OsAAO5-1F	CAAAGCCATCGAGATACTACGGTCAGATGG	crOsAAO1-1 CAPS off-target	
	OsAAO5-1R	CGACTTTCTTGATTGGTTTCCGACTGGTT	crOsAAO1-1 CAPS off-target	
		Tar-Cpf1-crRNA_DL-1F	AGATgtcttttgggtagCTGCAGGTGG	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_DL-1R	AAAACCAACCTGCAGctaccocaaagac	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_DL-2F	AGATgGACCTTGCACTGACTGCAGGAG	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_DL-2R	AAAACCTCTGCAGTCAAGTCAAGTCCc	cloning of crRNA into <i>Bbs</i> I site
		Tar-Cpf1-crRNA_ALS-2F	AGATccaacatacagattatagaTTAAT	cloning of crRNA into <i>Bbs</i> I site
	Tar-Cpf1-crRNA_ALS-2R	AAAAATTAAtctataatctgtatgttgg	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_ALS-1F	AGATACTCTTCTTTGTTACACGGACTG	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_ALS-1R	AAAACAGTCCGTGTAACAAGAAGAGT	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NCED1-1F	AGATCCCAAGGCCATTGGGGAGCTCCAT	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_NCED1-1R	AAAAATGGAGCTCCCAATGGCCTTGGG	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_AAO-1F	AGATGCAATGCTGTGCATATGTTAAT	cloning of crRNA into <i>Bbs</i> I site	
	Tar-Cpf1-crRNA_AAO-1R	AAAAATTAACATATGACACAGCATTCG	cloning of crRNA into <i>Bbs</i> I site	
	OsU6-2F	TGCTGGAATTGCCCTTGGATCATGAACCAA	crRNA sequence check	

Figure S1.

crNtSTF-1



crNtSTF-2



crNtSTF-3



Figure S2.

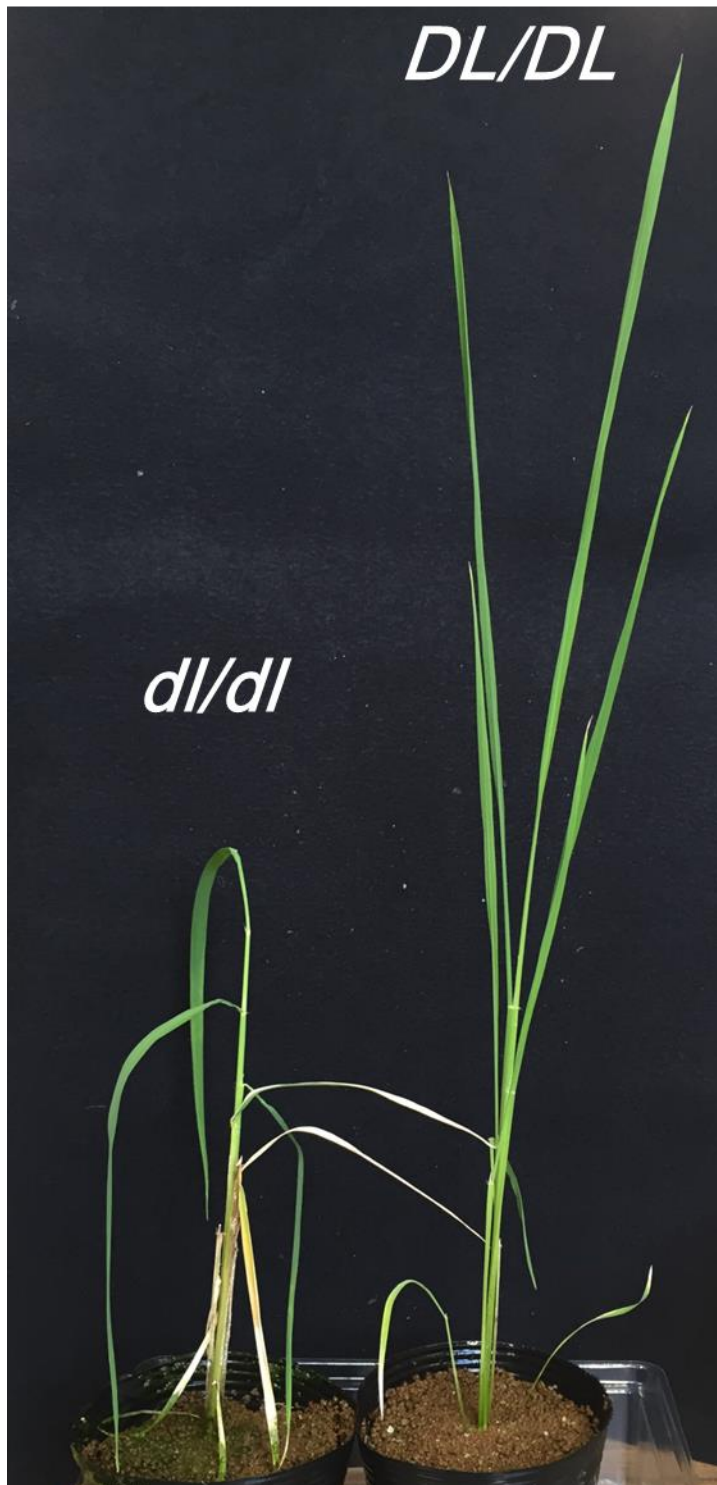


Figure S3.

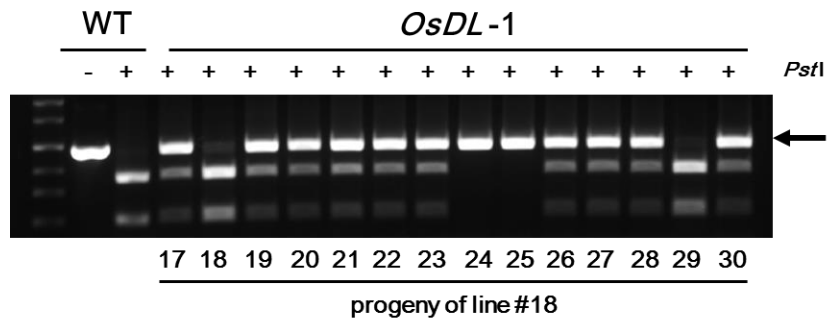
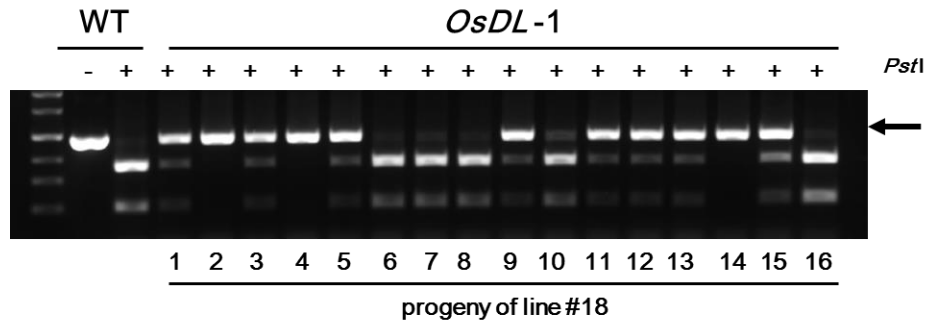


Figure.S4

