

Harnessing CRISPR/Cas9 for Enhanced Disease Resistance in Hot Pepper: A Comparative Study on *CaMLO2*-Gene-Editing Efficiency across six cultivars

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Supplementary Data

Table S1. Primers used in Sanger sequencing or targeted deep sequencing or sgRNA synthesis. The primary primer pair used for targeted deep sequencing was the same as that used for Sanger sequencing analysis.

Primer Name	Sequence
<i>CaMLO2</i> F	5'-ATGGCTAAAGAACGGTCGAT-3'
<i>CaMLO2</i> R	5'-ATGGAGCTGGTGTATTGCAT-3'
<i>CaMLO2</i> F1 (primary F)	5'-TGGGATTCATATCATTTGTGTG-3'
<i>CaMLO2</i> R1 (primary R)	5'-CCGAATGTGTCTCAGCCTTT-3'
sgRNA1 (without PAM)	5'-ACATCTTCATCTGCCTTACA-3'
sgRNA2 (without PAM)	5'-TGATGACCCTTGTTTACAAA-3'

D21101			D21102			E21301		
CaMLO2 sgRNA 1 Locus			CaMLO2 sgRNA 1 Locus			CaMLO2 sgRNA 1 Locus		
1st	CCTTGTGAAGGCAGATGAAGATGTCAA	WT	1st	CCTTGT-AAGGCAGATGAAGATGTCAA	WT	1st	CCTTGTGAAGGCAGATGAAGATGTCAA	WT
	CCTTGT---GCAGATGAAGATGTCAA	-2		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT---GCAGATGAAGATGTCAA	-3
	CCTTGT-----AGATGAAGATGTCAA	-5		CCTTGTaAAGGCAGATGAAGATGTCAA	+1		CCTTGT--AGGCAGATGAAGATGTCAA	-1
	CCTTGT-AGGCAGATGAAGATGTCAA	-1		CCTTGT-----AGATGAAGATGTCAA	-5		CCTTGT--GGCAGATGAAGATGTCAA	-2
2nd	CCTTGT----CAGATGAAGATGTCAA	-4	2nd	CCTTGT----GCAGATGAAGATGTCAA	-3	2nd	CCTTGT-AGGCAGATGAAGATGTCAA	-1
	CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT---GCAGATGAAGATGTCAA	-3
	CCTTGT-AGGCAGATGAAGATGTCAA	-1		CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT--GGCAGATGAAGATGTCAA	-2
3rd	CCTTGT---GCAGATGAAGATGTCAA	-3	3rd	CCTTGT---GCAGATGAAGATGTCAA	-3	3rd	CCTTGT-AGGCAGATGAAGATGTCAA	-1
	CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGTA--GCAGATGAAGATGTCAA	-2
	CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT---GCAGATGAAGATGTCAA	-3
4th	CCTTGT--GGCAGATGAAGATGTCAA	-2	4th	CCTTGT--GGCAGATGAAGATGTCAA	-2	4th	CCTTGT---GCAGATGAAGATGTCAA	-3
	CCTTGT-----TGAAGATGTCAA	-8		CCTTGT-----TGAAGATGTCAA	-9		CCTTGT-AGGCAGATGAAGATGTCAA	-1
	CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT-----AGATGAAGATGTCAA	-5		CCTTGT--GGCAGATGAAGATGTCAA	-2
5th	CCTTGT--GGCAGATGAAGATGTCAA	-2						
	CCTTGT---GCAGATGAAGATGTCAA	-3						
	CCTTGT-AGGCAGATGAAGATGTCAA	-1						
CaMLO2 sgRNA 2 Locus			CaMLO2 sgRNA 2 Locus			CaMLO2 sgRNA 2 Locus		
1st	GTATGATGACCCCTGTTTACAAAGG	WT	1st	GTATGATGACCCCTGTTTACAAAGG	WT	1st	GTATGATGACCCCTGTTTAC-AAAAGG	WT
	GTATGATGACCCCTGT---CAAAAGG	-3		GTATGATG-----CAAAAGG	-11		GTATGATGACCCCTGTTTAC--AAAGG	-1
	GTATGATGACCCCTGTTTAC-AAAGG	-1		GTATGATGACCCCTGTTTAC-AAAGG	-1		GTATGATGACCCCTGTTTACaAAAAGG	+1
2nd	GTATGATGACCCCTGTTTAC-AAAGG	-1						
E21302			F21201			J21401		
CaMLO2 sgRNA 1 Locus			CaMLO2 sgRNA 1 Locus			CaMLO2 sgRNA 1 Locus		
1st	CCTTGT-AAGGCAGATGAAGATGTCAA	WT	1st	CCTTGT-AAGGCAGATGAAGATGTCAA	WT	1st	CCTTGT-AAGGCAGATGAAGATGTCAA	WT
	CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT---GCAGATGAAGATGTCAA	-3
	CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT--GGCAGATGAAGATGTCAA	-2
	CCTTGT---GGCAGATGAAGATGTCAA	-2		CCTTGT-----CAGATGAAGATGTCAA	-4		CCTTGT--AGGCAGATGAAGATGTCAA	-1
2nd	CCTTGTaAAGGCAGATGAAGATGTCAA	+1	2nd	CCTTGT-----AGATGAAGATGTCAA	-5	2nd	CCTTGT---GGCAGATGAAGATGTCAA	-2
	CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT-A--GCAGATGAAGATGTCAA	-2		CCTTGT-----AGATGAAGATGTCAA	-5
	CCTTGT-----CAGATGAAGATGTCAA	-4		CCTTGT---GCAGATGAAGATGTCAA	-3		CCTTGT--AGGCAGATGAAGATGTCAA	-1
3rd	CCTTGT---GCAGATGAAGATGTCAA	-3	3rd	CCTTGT---GCAGATGAAGATGTCAA	-3	3rd	CCTTGTaAAGGCAGATGAAGATGTCAA	+1
	CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT--AGGCAGATGAAGATGTCAA	-1
	CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT---GGCAGATGAAGATGTCAA	-2
4th	CCTTGTaAAGGCAGATGAAGATGTCAA	+1	4th	CCTTGT---GCAGATGAAGATGTCAA	-3	4th	CCTTGT--AGGCAGATGAAGATGTCAA	-1
	CCTTGT-----CAGATGAAGATGTCAA	-4		CCTTGT--AGGCAGATGAAGATGTCAA	-1		CCTTGT---GGCAGATGAAGATGTCAA	-2
	CCTTGT---GGCAGATGAAGATGTCAA	-2		CCTTGT--GGCAGATGAAGATGTCAA	-2		CCTTGT-----GCAGATGAAGATGTCAA	-3
5th	CCTTGTaAAGGCAGATGAAGATGTCAA	+1	5th	CCTTGT---GCAGATGAAGATGTCAA	-3			
	CCTTGT-----CAGATGAAGATGTCAA	-3		CCTTGTaAAGGCAGATGAAGATGTCAA	+1			
	CCTTGT-----GATGAAGATGTCAA	-7		CCTTGT--GGCAGATGAAGATGTCAA	-2			
CaMLO2 sgRNA 2 Locus			CaMLO2 sgRNA 2 Locus			CaMLO2 sgRNA 2 Locus		
1st	GTATGATGACCCCTGTTTAC---AAAAGG	WT	1st	GTATGATGACCCCTGTTTAC-AAAAGG	WT	1st	GTATGATGACCCCTGTTTACAAAGG	WT
	GTATGATGACCCCTGTTTACcggAAAAGG	+3		GTATGATGACCCCTGTTTAC--AAAGG	-1		GTATGATGACCCCTGT---CAAAAGG	-3
	GTATGATGACCCCTGTTTAC---AAAGG	-1		GTATGATGACCCCTGTTTACaAAAAGG	+1		GTATGATGACCCCTGTTTAC-AAAGG	-1
2nd	GTATGATGACCCCTGTTTAC---AAAGG	-1	2nd	GTATGATGACCCCT---C-AAAAGG	-6	2nd	GTATGATGACCCCTGTTTAC-AAAGG	-1
	GTATGATGACCCCTGTTTACa--AAAAGG	+1		GTATGATGACCCCT---C-AAAAGG	-7			
	GTATGATGACCCCTGTTTACa--AAAAGG	+1		GTATGATGACCCCTGT---C-AAAAGG	-3			
3rd	GTATGATGAC---C---AAAAGG	-9	3rd	GTATGATGACCCCTGT---C-AAAAGG	-3			
	GTATGATGACCCCTGT---C---AAAAGG	-3		GTATGATGACCCCTGTTTAC-AAAGG	-1			
	GTATGATGACCCCTGTT---AAAAGG	-3						

Figure S1. The top 3-ranked *CaMLO2* editing patterns of all biological replicates of six commercial hot pepper cultivars. Red letter, PAM sequences; blue letter, CRISPR target (sgRNA) sequence; red hyphen (-), deleted nucleotide; red small letters (+), inserted nucleotide.

Table S2. Primers used in targeted deep sequencing for off target (OT) analysis.

Primer Name	Sequence
OT1 F	5'-GTCATATGGGTGCATAGTGAGAC-3'
OT1 R	5'-ATCACGATCAGCAGTCCATGA-3'
OT1 F1	5'-TTCATTAACCTATCGCACTCC-3'
OT1 R1	5'-GGTCCCAGAACAGTATCCA-3'
OT2 F	5'-CACTATCTCAAGAAGCTCTGCC-3'
OT2 R	5'-AATTGATGGGAAGAAGAGTGTGT-3'
OT2 F1	5'-AAGAACCAAACACATAGAGAAGA-3'
OT2 R1	5'-CCTACTTGAGCTGGAAACC-3'
OT3 F	5'-TGTGGTTCAGAAATTGTGCGAG-3'
OT3 R	5'-GGGTCAAAAAGCACGTCACC-3'
OT3 F1	5'-GCAATCCTCCAAATAACCCCT-3'
OT3 R1	5'-TGAATCCTCAAAACCCAAGAA-3'
OT4 F	5'-GAGTTTGTAGTGGTTTAAATGGT-3'
OT4 R	5'-CCACGGGATATAGGGGTCTA-3'
OT4 F1	5'-CATGCAAGGTAAACGGGAAT-3'
OT4 R1	5'-GCAAGCAATGTCTTTAGTAAACC-3'
OT5 F	5'-CACTAAGCCCAAGTCTGATGTAC-3'
OT5 R	5'-GCTCATCCACCAAGTACTCTC-3'
OT5 F1	5'-ACGCTCTCAGATTGGCTAG-3'
OT5 R1	5'-CGAAATAGGCCTCACACGG-3'
OT6 F	5'-GAAACGAGGAGAAGAATGGATGC-3'
OT6 R	5'-CACATTCTTTTGCTGTCTCTTCC-3'
OT6 F1	5'-TGGTTCCACCACAAGGAGAT-3'
OT6 R1	5'-ACTGAAGGCGCTTGTAC-3'

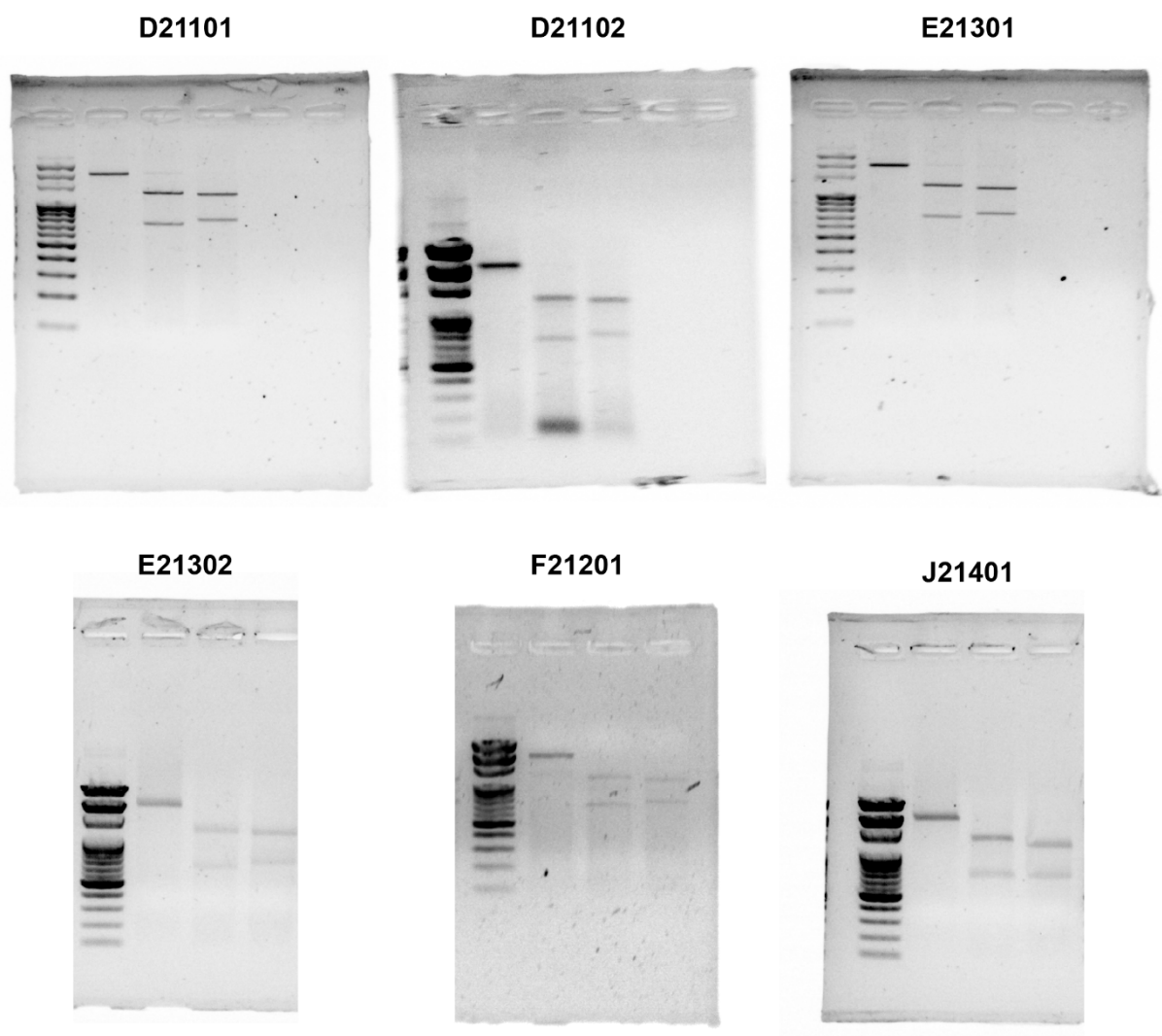


Figure S2. The original Gels of Figure 2.