

Supporting information

Establishment of a Genome Editing Tool Using CRISPR-Cas9 in *Chlorella vulgaris* UTEX395

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atgacagtgtctcctggcaggcgaggactcagccacgcgcagcgggtctgcaggagcagcaatgaaagccaatggc

T2 T3

tacagcacagaggcaccagcagcatcgcccaagcaccgcgcgcagtgaggagccgctgcagcccgccactgacgagtaacaagctgcacctgcccgtcaccgaagtgtggacgcagacaagggcaccgaagacgagtggaatccccgtgtagctctcatactgcggctcaagactgcgagccaggcgccggcggtgggtgctgctgggtccccgtgcaacctgtgctcatgcctttctgcatgggtgcagcagctctgccgcgtgtgtcgcccgagattgtgcatctttgcagcagatgcattcctcctctgcaacctcctggccccctcacctgccggcgctgcaaacacgtcgcacctccttgcatggctgcacagcctgtgtcgctcttgccactcaacgcctccccctgctgccctgccctgcagcaccacagaccttgtgcggctcacaggcgccgcatcccttcaacgtcgagcccatcccgctccgcctctttgagtcgtacatcacaccacctccttgcaactatgtacgcaaccacggagctgtaccgcagattaaagtgggaggagcaccgcctggcagtgcaacggtgagggggcgctgtatggctagctcatagccaggtagcgtgtctagctggctagctatcgtgtccagctagcctgcaagtgtatatggctagcatggctgggcgggcagcctccacaacagcaggcagtgagccagccagggtgggtcttttaggggtttttttgtaggggttttaggggtctacagacaggaaggggagcctccctctcttgcatcctccttgccgtgactgctgctcgctcggtgctctagccccgtcggtcagcgatgtctcaccttgcgtgccctgtttgtttgcccttgttttcccgcaggcctgggtttctgcgcccaccacattcacaatggacgacatcctcgcgatgcctagtgttgacgtgacctgtacactgacctgcgcagggtgcagtggttggaggtgggttggcgctgccaggctgcctgttgctgctgttttgctgccatcctgttttgcttttgccagcaagtacagactaacgctccagcattgccaaaccacactggcccgctgcattgtttgttcaaacgggttgccctcctcctgctgtcagctgcgggtactgcggcaccgccttgccactgagcactcctgcctttctcctgtactgcaggcaaccgcgcgaaggaggagaacatggtgaagcagacgataggcttcaactggggggcctcgggcacaggctgctccacctggacaggcgtgcgcgtgagcgacttgctgcagcgctgcgggatgaagacgcaccaaggaggcgcgccacgctgtgcttccgaggcccaaggggtgcgtgctgcagcgtgccagctgccgcaggcagcttacaatgcgcgcagctgcgcagcggagcctgcgggtgtcgtattgagtagcgttcggcatgctgctcctctcccgcatttgtgacttggcgggcgcgactgctcgcaattgtttcgtcgctcgatcactcatgctgctgcatctcagcgtctcttcttgtcctttcttttctgttttgaggcgagctgcccgaaggagacgcgcagctacggcaccagcgtcacctgggtaaaggctatggaccagcctctgacgtcctcgcatacaagcagaacggccgctgctgacacccgaccacggctaccgcgtgcggctcatcattccaggctacatcgccggcgcatgatcaagtgttgagcaggcctggctgtgctgtttaaaagcctggccacaatagcgggagccgacggccatgctctcctctgctctggagacccccctcctgcccttgccccctcctgcccttgctgtcatgtttcctgcccttgcttgcttcaactcattctcactacttctttcttatctttcttcttcttcttgcaggctggaggagatcacggtcaccgaggtggagagccagaactactaccacttccatgacaacagagtgtgccctcgacgttgatgaggcgctggccaaactccgaagggtgggcgcgcgcagggtgtgtgtgcgtgtatggttgtacgggttcaggcgtgttaggggtagggtgcaggagtctggagggtgtgcgccagggtacagtgctgtgctgggtgtgtgtgtgtgttgctgggtgggcattgcagctacatcagtgggcgggcgaccagggtgggtgcaagcgtgtgctggtaaatggtggcggtgcgtggaggcgttgccggctcagtgataactgcacgtcgtctgcacgtgcccttcgtttctttttgctggctcaaggtcagcctctgccaccgcacctctggctgcaggctgggtggtacaagccggacttcatcatcaatgacctcaatgtccagtcagccataggctacccggcacatgaagagggtgttgccccctcgcgggccggcaccacgcgttacgaggctacgcgtactgcgggtgagagccgcgtgtacagcacacctgcgctacttactgcacaacgccggccggggtgcgcgcacaagcagcagctggcgttcagcctgcagttgcccacccggccatgcgcttggcgctctctctctccatgctgctcgtcgtcatctccccctgcgcgtcatttcccccccgccaaggaacaagctctgctcctttgcaggcaacggccaacaagatcattcggtgcgagggttcgctggagcagcggcaagagctggcggttgggtctgtgacgcacgaggggcaacctactgagtagcggaagcactggggctgggtgtggtggagcttggagggtgcccatgggtgagttagagtgtaaactagccacaggcgctgggtgatgtgttgccgcgtgcgtacgaacacaccgcgcttgaccagtcgtgcgtgcgcagcagcacttcaaacgcatcaagctgggtgcgtgctttgctggaggctagcctgcttgcctgccctgcttgccctgcgcctcacctgggcgctgaggaccccgctgcttgcgtgccgcgctccttccccaccggctgtgctgcaagctgagctgcttaccactcctgaaatcatttgccgagcctgggacagctccatgaacacacagcccaacacggttccactgggtgtgctgctcctccccacctccccctccatgctgctgctgttgctcacctgcctggcttcagctgcccagcgcctcagcctgcgacactccgtgcggcaattggctgctgcccggaccacttgccagccgacggcactttgagcctgggtcgctgttcaccagtcacgcgcgtctgttctgtttccacctgagcctcgcttgctgaaagctcctcattcatttcatctctcacctttcctccacctcacctgtcctgcaggaaacgtgatggggatgatgaacaactgctgctatcgctcaagatccacccccgcagaccaccgacggccgctttgcgtgcaagtttgagcaccaccacatttgccggccccactgtcggcggctggatgaaccgagcagaggacgtggcgggcgggcgagcagtgaagggttaagggggccggggccgctgtgctgctggacactgtgctgctcggcacatgaaccacacagcagaggcatgtgtgactaattgtgcagctgctgccgtgcgtcggttgtctggcaacgcaagcaaaagcgttttccatgtttgagcacgcctgcttcccgtcctgcccctctgcgcgtgctgaaagtgtgctgcccagatgctttcacaccatcaaccaacccgcaacctgtagctgctggaacttcttgttgcgtgcagggtggcgccgcccgcgcagaggtgagtgcccaagagcttaccatggcagagtgagcagcagaccatggagagcgcgtggtttgttgttaggaaggtgtacgacgcaaacaccttctgaaagaccacccgggtgagccattgctgttttgttgacactgctgctgtccccgcaaaagtacagccgatacatacttqgttatgcatccttgaccacctcctgctgctgcttccccgc

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gtttgcctgctgccccacctcgagaagctgggctacaagccggagcagtgcattcaattttga

Figure S1. Sequence of the nitrate reductase gene of *C. vulgaris* UTEX395. The NR sequence is 7,039 bp long and contains 19 exons, indicated in yellow. Target sequences for gene editing are highlighted by blue boxes.

Figure S2

T1

atgggggctgacccgcgcaccgcgctcatctcagacagcatccgcatcatccccgacttcccaaaggtgaggcgg
aaagtgatgatggtgcccgtcatgcattgtacatcgcagactcaacagcaatcagactcgcctcttctgtgtccag
gcagggatcatgtttcaagatgtgaccaccattcttctggatccggtcgccttcaaacacactgttgacatgctg

T2

catgagcggtaacaggggacccaaaatccatcgggttgacaggtggggcggtgtggcaggcattgctaaaacagctta
tggcgcttagtcaaacctgcctactgaccaccaaccttccctggtaaccttttgtcacttgtgctgccgcaggga
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cctgggaagctgccaggagacgtgctgtcagcagactacgtgacggaatacagcaccgaccgcatagagatgcat

T3

gtgggggcagtgcggcaaggacagcgagtgtgtgtgtagacgacctgattgccacggggggcacgctacgtgag
tgctacagatatctcacacgggctgccatcacccaactggcagcacattgccagcatgtttgtctttgaatgac
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cataattgagttgcccagactcaagggcagggagaaagctggagggcctgccactatttgtgctggtagagaagga
gggcttgtga

Figure S2. Sequence of the adenine phosphoribosyltransferase gene of *C. vulgaris* UTEX395. The *APT* sequence is 835 bp long and contains four exons, indicated in yellow. Target sequences for gene editing are highlighted by blue boxes.

Figure S3

atggactacaaggatcacgacggcgactacaaggaccacgacatcgactataaggacgacgacgacaagatggcc
cccaagaagaagcgaaggtgggcatccacggggtgccgctgccgacaagaagtacagcattggcctggacatc
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accagcacaaggaggtgctggacgccaccctgatccaccagtccatcaccggcctgtacgagacgcgcattgac
ctgagccagctggggggcgataagcggccccgcgccaccaagaaggcgggccaggccaagaagaagaagtga

Figure S3. Sequence of the codon-optimized *Streptococcus pyogenes cas9* synthesized in this study.

Figure S4

A

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1 atg ggg gct gac ccg cgc acc gcg ctc atc tca gac agc atc cgt atc atc ccc gac ttc 60
1 M G A D P R T A L I S D S I R I I P D F 20

61 cca aag gca ggg atc atg ttt caa gat gtg acc acc att ctt ctg gat ccg gtc gcc ttc 120
21 P K A G I M F Q D V T T I L L D P V A F 40

121 aaa cac act gtt gac atg ctg cat gag cgg tac cag ggg acc aaa atc gat gtg gtt gca 180
41 K H T V D M L H E R Y Q G T K I D V V A 60

181 gga ttt gag gca aga ggg ctc ata ttt ggt gct ccc ctg gcc atc gca ctg ggg tgc gcg 240
61 G F E A R G L I F G A P L A I A L G C A 80

241 ttt gtg cca ctg cgc aag cct ggg aag ctg cca gga gac gtg ctg tca gca gac tac gtg 300
81 F V P L R K P G K L P G D V L S A D Y V 100

301 acg gaa tac agc acc gac cgc ata gag atg cat gtg ggg gca gtg cgg caa gga cag cga 360
101 T E Y S T D R I E M H V G A V R Q G Q R 120

361 gtg ctg ctg gta gac gac ctg att gcc acg ggg ggc acg cta cga gcg ggt gtg gag ctg 420
121 V L L V D D L I A T G G T L R A G V E L 140

421 gtg cag aag gcg ggc ggg cag gta gtc gaa gcg gca tgc ata att gag ttg ccc gag ctc 480
141 V Q K A G G Q V V E A A C I I E L P E L 160

481 aag ggc agg gag aag ctg gag ggc ctg cca cta ttt gtg ctg gta gag aag gag ggc ttg 540
161 K G R E K L E G L P L F V L V E K E G L 180

541 tga
*
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B

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1 atg ggg gct gac ccg cgc acc gcg ctc atc tca gac agc atc cgt atc atc ccc gac ttc 60
1 M G A D P R T A L I S D S I R I I P D F 20

61 cca aag gca ggg atc atg ttt caa gat gtg acc acc att ctt ctg gat ccg gtc gcc ttc 120
21 P K A G I M F Q D V T T I L L D P V A F 40

121 aaa cac act gtt gac atg ctg cat gag cgg tac cag ggg acc aaa atc gat gtg gtt gca 180
41 K H T V D M L H E R Y Q G T K I D V V A 60

181 gga ttt gag gca aga ggg ctc ata ttt ggt gct ccc ctg gcc atc gca ctg ggg tgc gcg 240
61 G F E A R G L I F G A P L A I A L G C A 80

241 ttt gtg cca ctg cgc aag cct ggg aag ctg cca gga gac gtg ctg tca gca gac tac gtg 300
81 F V P L R K P G K L P G D V L S A D Y V 100

301 acg gaa tac agc acc gac cgc ata gag atg cat gtg ggg gca gtg cgg caa gga cag cga 360
101 T E Y S T D R I E M H V G A V R Q G Q R 120

361 gtg ctg ctg gta gac gac aag atg tga cca cca ttc ttc tgg atc cgg tcg cct tca aac 420
121 V L L V D D K M * 128
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C

```
1 atg ggg gct gac ccg cgc acc gcg ctc atc tca gac agc atc cgt atc atc ccc gac ttc 60
1 M G A D P R T A L I S D S I R I I P D F 20

61 cca aag gca ggg atc atg ttt caa gat gtg acc acc att ctt ctg gat ccg gtc gcc ttc 120
21 P K A G I M F Q D V T T I L L D P V A F 40

121 aaa cac act gtt gac atg ctg cat gag cgg tac cag ggg acc aaa atc gat gtg gtt gca 180
41 K H T V D M L H E R Y Q G T K I D V V A 60

181 gga ttt gag gca aga ggg ctc ata ttt ggt gct ccc ctg gcc atc gca ctg ggg tgc gcg 240
61 G F E A R G L I F G A P L A I A L G C A 80

241 ttt gtg cca ctg cgc aag cct ggg aag ctg cca gga gac gtg ctg tca gca gac tac gtg 300
81 F V P L R K P G K L P G D V L S A D Y V 100

301 acg gaa tac agc acc gac cgc ata gag atg cat gtg ggg gca gtg cgg caa gga cag cga 360
101 T E Y S T D R I E M H V G A V R Q G Q R 120

361 gtg ctg ctg gta gac gac ctg att gcc acg ggg ggc acg cta cga ttt gag gca aga ggg 420
121 V L L V D D L I A T G G T L R F E A R G 140

421 ctc ata ttt ggt gct ccc ctg gcc atc gca ctg ggg tgc gcg ttt gtg cca ctg cgc aag 480
141 L I F G A P L A I A L G C A F V P L R K 160

481 cct ggg aag ctg cca gga gac gtg ctg tca gca gac tac gtg acg gaa tac agc acc gac 540
161 P G K L P G D V L S A D Y V T E Y S T D 180

541 cgc ata gag atg cat gtg ggg gca gtg cgg caa gga cag cga gtg ctg ctg gta gac gac 600
181 R I E M H V G A V R Q G Q R V L L V D D 200
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601 ctg att gcc acg ggg ggc acg cta cga gcg ggt gtg gag ctg gtg cag aag gcg ggc ggg 660
201 L I A T G G T L R A G V E L V Q K A G G 220

661 cag gta gtc gaa gcg gca tgc ata att gag ttg ccc gag ctg aag ggc agg gag aag ctg 720
221 Q V V E A A C I I E L P E L K G R E K L 240

721 gag ggc ctg cca cta ttt gtg ctg gta gag aag gag ggc ttg tga 765
241 E G L P L F V L V E K E G L * 254

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Figure S4. Sequence analysis of the adenine phosphoribosyltransferase gene of *C. vulgaris* UTEX395. (A) Gene and protein sequences of wild-type *APT*. (B) Edited mRNA sequence of *APT* gene in *apt1*. (C) Edited mRNA sequence of *APT* gene in *apt2*. Edited sequences do not show the expression of right amino acid of *APT*. Red alphabet indicates the stop codon.

Table S1. Primer sequences for the construction of the *cas9* expression vector.

Name	Sequence (5' → 3')
sgRNA NR-F1	ATATATGGTCTCGattgAGCCAATGGCTACAGCACAGGTTTTAGAGCTAGAAATAGC
sgRNA NR-R1	ATTATTGGTCTCGCACTCGGCGGCGGGTGCTTGTCACCAGCCGGGAATC
sgRNA NR-F2	ATATATGGTCTCGagtGTTTTAGAGCTAGAAATAGC
sgRNA NR-R2	ATTATTGGTCTCGaaacAGTGCGGCAAGGCGCCGGTGTCACCAGCCGGGAATC

* Gray box: sgRNA sequence

* Red box: tRNA sequence

* Green box: tracrRNA sequence

* Red characters: GGTCTC, *Bsa* I recognition sequence

Table S2. Primer sequences for Sanger sequencing of edited genes.

Name	Sequence (5' → 3')
<i>CvNR</i> -F	CAACTCCTCCCTCATACTCCTCGCAC
<i>CvNR</i> -R	CCACTCGTCCTTGGTGCCCTTGTC
<i>CvAPT</i> -F	CCGTCCACATCCAGTCAACA
<i>CvAPT</i> -R	TGAGCTCGGGCAACTCAATT