

## SEQUENCES USED IN THE STUDY

### ❖ gRNA expression cassettes and donor sequences for SIANT1 locus (shown in the Figures 2 and 3)

- U6-crR1.20<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATTAGAAGGCTCTCTACAAGTTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; light blue font: LbCas12a\_gRNA1, 20nt;  
black G: transcription start; black TTTTTT: termination sequence.

- U6-crR1.23<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATTAGAAGGCTCTCTACAAGTTGGTTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; blue font: LbCas12a\_gRNA1, 23nt; black G:  
transcription start; black TTTTTT: termination sequence.

- U6-crR3.20<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATCCAATCGAGGCTGGCAGGATTTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; purple font: LbCas12a\_gRNA3, 20nt; black  
G: transcription start; black TTTTTT: termination sequence.

- U6-crR3.23<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATCCAATCGAGGCTGGCAGGATAGGTTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; orange font: LbCas12a\_gRNA3, 23nt; black  
G: transcription start; black TTTTTT: termination sequence.

- Dual U6-crR1-3.20<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATTAGAAGGCTCTCTACAAGTTTAAATTTCTACTAAGTGTAGATCCAATCGAG  
GCTGGCAGGATTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; light blue font: LbCas12a\_gRNA1, 20nt;  
purple font: LbCas12a\_gRNA3, 20nt; black G: transcription start; black TTTTTT: termination  
sequence.

- Dual U6-crR1-3.23<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATTAGAAGGCTCTCTACAAGTTGGTTAATTTCTACTAAGTGTAGATCCAATC  
GAGGCTGGCAGGATAGGTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; blue font: LbCas12a\_gRNA1, 23nt; orange  
font: LbCas12a\_gRNA3, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-sgR2<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGGTAGAAGGCTCTCTACAAGTgttttagagctagaatagcaagttaaaataaggctagtcggttatcaactgaaaaagtgccac  
cgagtcggtgcTTTTTT

Red font: AtU6 promoter; green font: SpCas9 scaffold; purple font: SpCas9\_gRNA2, 20nt; black G:  
transcription start; black TTTTTT: termination sequence.

- U6-sgR3<sup>ANT1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
 TTGCCAATCGAGGCTGGCAGGATgttttagagctagaatagcaaggttaaataaggctagtcggttatcaacttgaaaaagtgcca  
 ccgagtcggtgcTTTTTT

Red font: AtU6 promoter; green font: SpCas9 scaffold; blue font: SpCas9\_gRNA3, 20nt; black G:  
 transcription start; black TTTTTT: termination sequence.

- ANT1D2 donor sequence

TTGGTCCCCAAGTACTTAAATTGGTCCCCAAGTACTTACCACAACACTTGTCTGGTGAGATTATTTAATGCTGAT  
 TAGATTAGACAAAAATTAATTAGTTTTGAGTAGTGCCGTAAGTGAAATAATTAGTCTCTTTTTAACTTAGAA  
 AATAGTTAATCCTTAGTATAAATAGTCAAATCACTGGAATGAAAAACAGTTTTTAATTTTTCCAAATTTGATT  
 CTGATACCATGTTAAATTCGTGGTTCAAATCACTGCAATGAAAAGAGCAATATTGTTAACTTTTTTAGGAA  
 AATCGAATTGATTTATAGTCAGTTGATATAGAGTGAATACATAAGGAACATATACAGTTGATACAATTGTATA  
 ATTCGTTACACCTAATACAAAGTGAACCCACAAGGAACATATACACTTAATATAATTGTATTCTTGATAC  
 AAACCAATTTTGTCTGTCTCTACTCTCTATTCAATTCGCTTACTCTTTTCTAATATGTAGCTATAA  
 ATCGTAATTAACAATACTATATCTCTAAATCTTATTAAGCTCAAATATGGTCATATTCGAAAAATCCTTT  
 TAAATATTGGTCCCCTCTCACGATTAATGATAGTTATAACTAACATTCAAATTTTAGTTGACTTGACATCTAAA  
 ACTTAAAAAATAGTACAAGTTAACTTTTTCTTTTTTAAAAAAGGAAATACTTGTATTTATTTTTTAATATAT  
 AGTTATATTTTGGTTATTTGAAAATACTTGATCTGTCATGTATGCTCAGTTAAATATCGTCACATTATAGAGAA  
 AAAAGTAATAGGAGAAAAAATTAATAATTTTCGAAAAATCAAATTTTTTTTTGATTGAAATGAAAGATG  
 GGTTTCCAATCGAGGCTGGCAGGATAGGTACATTGGGAAATTTGGATTGTGTGTTGAAAATGATTGTTCAA  
 TTTGGCTTTATAACATTTGTCGTTTATAAGGTGTAGAAGGCTCTCTACAAGTTGGTAGTTACAATTTAATACA  
 CCTGAATTCGGATCCGGAGCGGAGAATTAAGGGAGTCACGTTATGACCCCGCCGATGACGCGGGACAAGC  
 CGTTTTACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTGAGCCGCGGGTTTCTGGAGTTTAA  
 GAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAGTGCCTAAGGTCATATCAGCTAGCAAAT  
 ATTTCTGTCAAAAATGCTCCACTGACGTTCCATAAATCCCCTCGGTATCCAATTAGAGTCTCATATCACTCT  
 CCTATTTTACAACAATTACCAACAACAACAACAACAACAACATTACAATTACATTTACAATTACCATGGTT  
 GAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAA  
 CAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTGAGCGCAGGGGCGCCCGTTCTTTTTGTCAAGA  
 CCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGC  
 GTTCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCG  
 GGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGC  
 TGCATACGCTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAATCG  
 GATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAATGT  
 TCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCGTGACTCATGGCGATGCCTGCTTGCCGA  
 ATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATC  
 AGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGCGAATGGGCTGACCGCTTCTCGTGC  
 TTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTGACGAGTCTTCTGAGCGGGA  
 CTCTGGGGTTGCTAGAGTCTGCTTAAATGAGATATGCGAGACGCTATGATCGCATGATATTTGCTTTCAAT  
 TCTGTTGTGCACGTTGTAAAAAACCTGAGCATGTGTAGCTCAGATCCTACCGCCGGTTTCGGTTCATTCTAAT  
 GAATATATACCCGTTACTATCGTATTTTTATGAATAATTTCTCCGTTCAATTTACTGATTGTACCCTACTACT  
 ATATGTACAATATTAATAAATGAAAACAATATATTGTGCTGAATAGTTTATAGCGACATCTATGATAGAGCGCC

ACAATAACAAACAATTGCGTTTTATTATTACAAATCCAATTTTAAAAAAGCGGCAGAACCGGTCAAACCTAA  
AAGACTGATTACATAAATCTTATTCAAATTTCAAAGTGCCCCAGGGGCTAGTATCTACGACACACCGAGCGG  
CGAACTAATAACGCTCACTGAAGGGAACCTCCGTTCCCCGCCGGCGCATGGGTGAGATTCTTGAAGTTG  
AGTATTGGCCGTCCGCTCTACCGAAAGTTACGGGCACCATTCAACCCGGTCCAGCACGGCGGCCGGGTAACC  
GACTTGCTGCCCGAGAATTATGCAGCATTTTTTTGGTGTATGTGGGCCCAAATGAAGTGCAGGTCAAACCT  
TGACAGTGACGACAAATCGTTGGGCGGGTCCAGGGCGAATTTTGCGACAACATGTCGAGGCTCAGCCGCTGT  
CAGTCAACATGGTGGAGCACGACACTCTGGTCTACTCCAAAAATGTCAAAGATACAGTCTCAGAAGATCAAA  
GGGCTATTGAGACTTTTTCAACAAAGGATAATTCGGGAAACCTCCTCGATTCCATTGCCAGCTATCTGTAC  
TTCATCGAAAGGACAGTAGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCTATC  
ATCAAGATCTCTGCCGACAGTGGTCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGAAAAAGAA  
GAGGTTCCAACCAGTCTACAAAGCAAGTGGATTGATGTGACATCTCCACTGACGTAAGGGATGACGCACAA  
TCACACTATCCTTCGCAAGACCCTTCTCTATATAAGGAAGTTCATTTTATTGGAGAGGACACGCTCGAGTAT  
AAGAGCTCATTTTTACAACAATTACCAACAACAACAACAACAACAACATTACAATTACATTTACAATTATCG  
ATACAATGAAGTATATATAGACAATAAAAAAGTAGTATAATATATTATCAAATTATTATGAACAGTACATCTAT  
GTCCTCATTGGGAGTGAGAAAAGGTTTCATGGACTGATGAAGAAGATTTTCTTCTAAGAAAATGTATTGATAAG  
TATGGTGAAGGAAAATGGCATCTTGTCCATAAGAGCTGGTAACTATTAATTAATTAATTAATTAATTAATTAAT  
TTGTCTTCTGTCTCATTTTATTTGACGTTATTACGAATATCATCTGAAAATGTACGTGCAGGTCTGAATAGATG  
TCGAAAAAGTTGTAGATTGAGGTGGCTGAATTATCTAAGGCCACATATCAAGAGAGGTGACTTTGAACAAGA  
TGAAGTGGATCTCATTTTGGAGGCTCATAAGCTCTTAGGCAACAGGCATGCAAGTTTATGTTTTGACAAAATTT  
GATTAGTATATATTATATATACGTGTGACTATTTTCATCTAAATGTTACGTTATTTTACGTAGATGGTCACTTATT  
GCTGGTAGACTTCCCGAAGGACAGCTAACGATGTGAAAACTATTGGAACACTAATCTTCTAAGGAAGTTA  
AATACTACTAAAATTGTTCTCGCGAAAAGATTAACAATAAGTGTGGAGAAATTAGTACTAAGATTGAAATTA  
TAAAACCTCAACGACGCAAGTATTTCTCAAGCACAATGAAGAATGTTACAAACAATAATGTAATTTTGGACGA  
GGAGGAACA

Blue font: 2x TALE binding site (scTALE ABL1 R); green font: upstream homologous arm; red font: pNOS-NptII-tOCS; orange font: CaMV 35S promoter; purple font: downstream homologous arm.

- ANT1D3 donor sequence

TTGGTCCCCAAGTACTTAAATTGGTCCCCAAGTACTTACCACAACACTTGTGCGGTGAGATTATTTAATGCTGAT  
TAGATTAGACAAAAATTAATTAGTTTTGAGTAGTGGCGTAAGTGTAATAATTAGTCTCTTTTTTAACTTAGAA  
AATAGTTTAATCCTTAGTATAAATAGTCAAATCACTGGAATGAAAAACAGTTTTTAAATTTTTCCAAATTTGATT  
CTGATACCATGTTAAATTCGTGGTTCAAATCACTGCAATGAAAAGAGCAATATTGTTAACTTTTTTTAGGAA  
AATCGAATTGATTTATAGTCAGTTGATATAGAGTGAATACATAAGGAACATATACAGTTGATACAATTGTATA  
ATTCGTTACATACCTTAATACAAAGTGAACCCACAAGGAACATATACACTTAATATAATTGTATTCTTGATAC  
AAACCAATTTTGTTCGTGTCTCTACTCTCTATTTCAATTTGCTTACTCTTTACTTTTTCTAATATGTAGCTATAA  
ATCGTAATTAACAATACTATATCTCTAAATCTTATTAAGCTCAAACACTATGGTCATATTGAAAAAATCCTTT  
TAAATATTGGTCCCCTCTCACGATTAATGATAGTTATAACTAACATTCAAATTTTAGTTGACTTGACATCTAAA  
ACTTAAAAAATAGTACAAGTTAACTTTTTCTTTTTTTAAAAAAGGAAATACTTGTATTTATTTTTTAAATATAT  
AGTTATATTTTTGGTTATTTGAAAATACTTGATCTGTCATGTATGCTCAGTTAAATATCGTCACATTATAGAGAA  
AAAAGTAATAGGAGAAAAAATTAATAATTTTCGAAAAATCAAATTTTTTTTTGATTGAAATGAAAGATG  
GGTTTCCAATCGAGGCTGGCAGGATAGGTACATTGGGAAATTTGGATTTGTGTGTTGAAAATGATTGTTCAA  
TTTGGCTTTTATAACATTTGTCGTTTATAAGAATTCGGATCCGGAGCGGAGAATTAAGGGAGTCACGTTATGA

CCCCGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAAGTACAGAACCGCAACGTTGAAGGAGCCACT  
GAGCCGCGGGTTTCTGGAGTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGC  
CTAAGGCTACTATCAGCTAGCAAATATTTCTGTCAAAAATGCTCCACTGACGTTCCATAAATCCCCTCGGTA  
TCCAATTAGAGTCTCATATTTACTCTCCTATTTTTACAACAATTACCAACAACAACAACAACAACAACATTAC  
AATTACATTTACAATTACCATGGTTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGTTGGGTGGAGAG  
GCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCA  
GGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCG  
GCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA  
CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCC  
ATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAAC  
ATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATC  
AGGGGCTCGCGCCAGCCGAACTGTTCCGAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCGTG  
ACTCATGGCGATGCCTGCTTGCCGAATATCATGGTGAAAATGGCCGTTTTCTGGATTCATCGACTGTGGCC  
GGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGCGGGC  
GAATGGGCTGACCGTTCCTCGTGCTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCT  
TCTTGACGAGTCTTCTGAGCGGGACTCTGGGGTTCGCTAGAGTCTGCTTAAATGAGATATGCGAGACGCCT  
ATGATCGCATGATATTTGCTTTCAATTCTGTTGTGCACGTTGTA AAAAACCTGAGCATGTGTAGCTCAGATCCT  
TACCGCCGGTTTCGGTTCATTCTAATGAATATATCACCCGTTACTATCGTATTTTTATGAATAATATTCTCGTTC  
AATTTACTGATTGTACCCTACTACTTATATGTACAATATTA AAAATGAAAACAATATATTGTGCTGAATAGGTTTA  
TAGCGACATCTATGATAGAGCGCCACAATAACAACAATTGCGTTTTATTATTACAAATCCAATTTTAAAAAAA  
GCGGCAGAACCGGTCAAACCTAAAAGACTGATTACATAAATCTTATTCAAATTTCAAAGTGCCCCAGGGGCT  
AGTATCTACGACACACCGAGCGGCGAACTAATAACGCTCACTGAAGGGA ACTCCGGTTCGCCGCGCGC  
ATGGGTGAGATTCCTGAAGTTGAGTATTGGCCGTCGCTCTACCGAAAGTTACGGGCACCATTCAACCCGGT  
CCAGCACGGCGGCGGGTAACCGACTTGCTGCCCCGAGAATTATGCAGCATTTTTTTGGTGTATGTGGGCCCC  
AAATGAAGTGCAGGTCAAACCTTGACAGTGACGACAAATCGTTGGGCGGGTCCAGGGCGAATTTTGCAGCAA  
CATGTGCGAGGCTCAGCCGCTGTCA GTCAACATGGTGGAGCACGACACTCTGGTCTACTCCAAAAATGTCAAAG  
ATACAGTCTCAGAAGATCAAAGGGCTATTGAGACTTTTTCAACAAGGATAATTTCCGGAAACCTCCTCGGATT  
CCATTGCCAGCTATCTGTCACTTCATCGAAAGGACAGTAGAAAAGGAAGGTGGCTCCTACAAATGCCATCAT  
TGCGATAAAGGAAAGGCTATCATTCAAGATCTCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGA  
GGAGCATCGTGAAAAAGAAGAGGTTCCAACCACGTCTACAAAGCAAGTGGATTGATGTGACATCTCCACTG  
ACGTAAGGGATGACGCACAATCACACTATCCTTCGCAAGACCCTTCTCTATATAAGGAAGTTCAATTCATTTG  
GAGAGGACACGCTCGAGTATAAGAGCTCATTTTTACAACAATTACCAACAACAACAACAACAACAACATTA  
CAATTACATTTACAATTATCGATACAATGAAGTATATATAGACAATAAAAAAGTAGTATAATATATTATCAAAT  
TATTATGAACAGTACATCTATGTCCTCATTGGGAGTGAGAAAAGGTTTATGGACTGATGAAGAAGATTTTCTT  
CTAAGAAAATGTATTGATAAGTATGGTGAAGGAAAATGGCATCTTGTTCCATAAGAGCTGGTAACTATTA  
TTAACTATCACGTTATTTTTATTTGTCTTTCTGTCTCATTTTATTTGACGTTATTACGAATATCATCTGAAAATGT  
ACGTGCAGGTCTGAATAGATGTCGAAAAGTTGTAGATTGAGGTGGCTGAATTATCTAAGGCCACATATCAA  
GAGAGGTGACTTTGAACAAGATGAAGTGGATCTCATTTTGAGGCTTCATAAGCTCTTAGGCAACAGGCATGC  
AAGTTTATGTTTTGACAAAATTTGATTAGTATATATTATATACGTGTGACTATTCATCTAAATGTTACGTTA  
TTTTACGTAGATGGTCACTTATTGCTGGTAGACTTCCCGAAGGACAGCTAACGATGTGAAAACTATTGGAA  
CACTAATCTTCTAAGGAAGTTAAATACTACTAAAATTGTTCTCGCGAAAAGATTAACAATAAGTGTGGAGAA

ATTAGTACTAAGATTGAAATTATAAACCTCAACGACGCAAGTATTCTCAAGCACAATGAAGAATGTTACAA  
ACAATAATGTAATTTGGACGAGGAGGAACA

Blue font: 2x TALE binding site (scTALE ABL1 R); green font: upstream homologous arm; red font:  
pNOS-NptII-tOCS; orange font: CaMV 35S promoter; purple font: downstream homologous arm.

- ANT1D4 donor sequence

TTGGTCCCAAGTACTTAAATTGGTCCCAAGTACTTACCACAACACTTGTCTGGTGAGATTATTTAATGCTGAT  
TAGATTAGACAAAAATTAATTAGTTTTGAGTAGTGGCGTAAGTGAAATAATTAGTCTCTTTTTAACTTAGAA  
AATAGTTTAATCCTTAGTATAAATAGTCAAATCACTGGAATGAAAAACAGTTTTTAATTTTTCAAATTTGATT  
CTGATACCATGTTAAATTCGTGGTTCAAATCACTGCAATGAAAAGAGCAATATTGTTAACTTTTTTAGGAA  
AATCGAATTGATTTATAGTCAGTTGATATAGAGTGAATACATAAGGAACATATACAGTTGATACAATTGTATA  
ATTCGTTCATACACTTAATACAAAGTGAACCCACAAGGAACATATACACTTAATATAATTGTATTCTTGATAC  
AAACCAATTTTGTTCGTGTCTCTACTCTCTATTTCAATTCGCTTGACTCTTACTTTTTCTAATATGTAGCTATAA  
ATCGTAATTAACAATACTATATCTCTAAATCTTATTAAGCTCAAATATGGTCATATTCGAAAAATCCTTT  
TAAATATTGGTCCCCTCTCACGATTAATGATAGTTATAACTAACATTCAAATTTAGTTGACTTGACATCTAAA  
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AGTTATATTTTTGGTTATTTGAAAATACTTGATCTGTATGCTCAGTTAAATATCGTCACATTATAGAGAA  
AAAAGTAATAGGAGAAAAAATTAATAATTTTCGAAAAATCAAATTTTTTTTTGATTGAAATGAAAGATG  
GGTTTCGAATTCGGATCCGGAGCGGAGAATTAAGGGAGTACGTTATGACCCCGCCGATGACGCGGGACA  
AGCCGTTTTACGTTTGGAACTGACAGAACCAGCAACGTTGAAGGAGCCACTGAGCCGCGGGTTTCTGGAGTTT  
AATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAGTCGCTAAGGTCACTATCAGCTAGCA  
AATATTTCTGTCAAAAATGCTCCACTGACGTTCCATAAATCCCTCGGTATCCAATTAGAGTCTCATATTCAC  
TCTCTATTTTTACAACAATTACCAACAACAACAACAACAACAACATTACAATTACATTTACAATTACCATGG  
TTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCAC  
AACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTGAGCGCAGGGGCGCCCGTTCTTTTTGTCAA  
GACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGG  
GCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGC  
CGGGGACAGGATCTCCTGTCTACCTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCG  
GCTGCATACGTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTA  
CGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGA  
GTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACTCATGGCGATGCCTGCTTGC  
GAATATCATGGTGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTA  
TCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGCGAATGGGCTGACCGCTTCTCGT  
GCTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTGACGAGTCTTCTGAGCGG  
GACTCTGGGGTTCGCTAGAGTCTGCTTAATGAGATATGCGAGACGCCTATGATCGCATGATATTTGCTTTC  
AATTCTGTTGTGACGTTGTAAAAACCTGAGCATGTGTAGCTCAGATCCTTACCGCCGGTTTCGGTTCATCT  
AATGAATATATCACCCGTTACTATCGTATTTTTATGAATAATATTCTCCGTTCAATTTACTGATTGTACCCTACTA  
CTTATATGTACAATATTAATAATGAAAACAATATATTGTGCTGAATAGTTTATAGCGACATCTATGATAGAGC  
GCCACAATAACAACAATTGCGTTTTATTATTACAAATCCAATTTAAAAAAGCGGCAGAACCGGTCAAACC  
TAAAAGACTGATTACATAAATCTTATTCAAATTTCAAAGTGCCCCAGGGGCTAGTATCTACGACACACCGAG  
CGGCGAACTAATAACGCTCACTGAAGGAACTCCGGTTCGCCGCGCGCATGGGTGAGATTCTTGAAG  
TTGAGTATTGGCCGTCCGCTCTACCGAAAGTTACGGGACCATTCAACCCGGTCCAGCACGGCGCCGGGTA

ACCGACTTGCTGCCCCGAGAATTATGCAGCATTNTTTTGGTGTATGTGGGCCCAAATGAAGTGCAGGTCAAA  
 CCTTGACAGTGACGACAAATCGTTGGGCGGGTCCAGGGCGAATTTTTCGACAACATGTGCGAGGCTCAGCCGC  
 TGTCAGTCAACATGGTGGAGCACGACACTCTGGTCTACTCCAAAATGTCAAAGATACAGTCTCAGAAGATCA  
 AAGGGCTATTGAGACTTTTCAACAAAGGATAATTTTCGGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGTC  
 ACTTCATCGAAAGGACAGTAGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCTA  
 TCATTCAAGATCTCTCTGCCGACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAGCATCGTGAAAAAG  
 AAGAGGTTCCAACCACGTCTACAAAGCAAGTGGATTGATGTGACATCTCCACTGACGTAAGGGATGACGCAC  
 AATCACACTATCCTTCGCAAGACCCTTCTCTATATAAGGAAGTTCATTTTCATTTGGAGAGGACACGCTCGAGT  
 ATAAGAGCTCATTTTTACAACAATTACCAACAACAACAACAACAACAACATTACAATTACATTTACAATTAT  
 CGATACAATGAAGTATATATAGACAATAAAAAAGTAGTATAATATATTATCAAATTATTATGAACAGTACATCT  
 ATGTCCTCATTGGGAGTGAGAAAAGGTTTCATGGACTGATGAAGAAGATTTTCTTCTAAGAAAATGTATTGATA  
 AGTATGGTGAAGGAAAATGGCATCTTGTTCCATAAGAGCTGGTAACTATTAATAACTATCACGTTATTTTT  
 ATTTGTCTTCTGTCTCATTTTATTTGACGTTATTACGAATATCATCTGAAAATGTACGTGCAGGTCTGAATAGA  
 TGTCGGAAAAGTTGTAGATTGAGGTGGCTGAATTATCTAAGGCCACATATCAAGAGAGGTGACTTTGAACAA  
 GATGAAGTGGATCTCATTTTGGAGCTTCATAAGCTCTTAGGCAACAGGCATGCAAGTTTATGTTTTGACAAAA  
 TTTGATTAGTATATATTATATATACGTGTGACTATTTTCATCTAAATGTTACGTTATTTTACGTAGATGGTCACTT  
 ATTGCTGGTAGACTTCCCGAAGGACAGCTAACGATGTGAAAACTATTGGAACACTAATCTTCTAAGGAAGT  
 TAAATACTACTAAAATTGTTCTCGGAAAAGATTAACAATAAGTGTGGAGAAATTAGTACTAAGATTGAAAT  
 TATAAACCTCAACGACGCAAGTATTTCTCAAGCACAATGAAGAATGTTACAACAATAATGTAATTTTGGAC  
 GAGGAGGAACA

Blue font: 2x TALE binding site (scTALE ABL1 R); green font: upstream homologous arm; red font: pNOS-NptII-tOCS; orange font: CaMV 35S promoter; purple font: downstream homologous arm.

❖ **gRNA expression cassettes and donor sequences for SIHKT1;2 locus (shown in the Supplemental Figure 9)**

- U6-crR1.20<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
 TTGtAATTTCTACTAAGTGTAGATACTATTACCACAGTATCAAATTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; light blue font: LbCas12a\_gRNA1, 20nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-crR1.23<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
 TTGtAATTTCTACTAAGTGTAGATACTATTACCACAGTATCAACTTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; blue font: LbCas12a\_gRNA1, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-crR2.20<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
 TTGtAATTTCTACTAAGTGTAGATCCTACAAATGAAAACATGATTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; orange font: LbCas12a\_gRNA2, 20nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-crR2.23<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATCCTACAAATGAAAACATGATGATTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffold; purple font: LbCas12a\_gRNA2, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-crR1-2.20<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATACTATTCACCACAGTATCAATAATTTCTACTAAGTGTAGATCCTACAAATG  
AAAACATGATTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; light blue font: LbCas12a\_gRNA1, 20nt; orange font: LbCas12a\_gRNA2, 20nt; black G: transcription start; black TTTTTT: termination sequence.

- U6-crR1-2.23<sup>HKT1;2</sup> expression cassette:

TGATCAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATACTATTCACCACAGTATCAACTTTAATTTCTACTAAGTGTAGATCCTACAA  
ATGAAAACATGATGATTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; blue font: LbCas12a\_gRNA1, 23nt; purple font: LbCas12a\_gRNA2, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- HKT1;2 donor (HKTD1) sequence

GGGAGACTCATGAACTGAAATGCATAGAATGCCTATTTTAAACACGTTTTTAAACAAATAATAAATAAGTTGTATA  
GCATCTTATAATCCACTAACTCTATGATAAGGAAATATCAGTGATCATTTGGCAAGAATTATTATATTCTAACT  
CCTTTTGGTACGTACCATATGTTGTTGGGATTAATTTAAATCATTGGTAAGAATTATTATATTCTAACTCCTTTT  
GATACGTACCATATGTTATTGGGATTAATTTAAAGCTTACACAATGTGTCTAGTATAGGCATTCCACTACACAA  
TTTTACTATAAATTGAACCCTTGAGTATTATAATAAACTTAAGAGACAAACAAATTTATTTACTAATGAAGT  
CATCACTTTCAATTTCTTTCTTTAAGATTATGATGAGTCTTAGGGTAAAGCCATTTTGGATTGAATTAGGGTATT  
TCACAACCCTTTCTATTGTTAGGTTTCTTAGCTTTGAATTATGTTTCAAACCTAGAACCCTACCGTCTTTTCGTCC  
TCAAACCTAGATGTTCTTTTCACTTCTGTTTCTTCAACCACAGTTTCTAGTATGTCCACTATTGAAATGGAAGT  
TTTCTCAAATGTTCAACTGTTTTTCTATGACCATATTGATGTTTCTTGGTGGGGAAGCTTTTACCTCTTTTCTTAGC  
CTTAAACTCATCAAGAATAAAGAAAAGCAAAGATAAATCTTTTAGTAACAAAGATTATGAGCTAGGGAATGTAA  
TAAACGTTGACAATAAGTTAGAAGATGTGATAATAATAAACCTATCGAAGATCATATTCATGATCATCACGA  
TGAAATTATAAAGATTAAATCGATAAAATTGTTGAGTAATGTGGTTTTTGGATATATTCTTGTGTTATTCTTCT  
TGGTTCATCGTTAGTTTCTCTATATAATAATCATCCCTAGCGCCAAACAAATCCTTGACCAAAAAGGCCTTAA  
TTTACATACTTTCTCTTATTTCACCACAGTATCAACTTTTGCAAGATTGTGGTTTCTTACCACAAATGAAAACAT  
GATGATTTTCAAGAAAAATTCAGGACTTCTTCTCATTCTTATCCCTCAAGTCCTTCTAGGGAACACTTTGTTTGC  
TCCTTGTTTACGCATCGTTATAATGTTCTTATGGAAAATCACAAGAGACATGAGTATGAGTATATTTTGAAGA  
ACTCAAATGTGTTGGATATTCACATATTTTTCCAAGTTATGAAACAATTGGTATTGCTATTACTGTTGTGGGA  
TTAATAGTATTTCAATTTGTTATGTTTTGTTTATTGGAGTGGAAATTCTGAAGGTACTTCTGGATTAAGTACTTAT  
GAGAAGATTGTGGGATCTTTGTTTGAAGTTGTGAATACAAGGCATGCTGGTCTATCTGATTTGATCTTTCAAC  
TTTTACTCCATCAATCTTAGTATTGTTTGCCTAATGATGTAAGTATTCTCTACAATCTATGTTCTTTTCGTGTTCT  
GTCTCAATTTACGTGACATATACTACTTCTTTGTTTTATATCTGTGTCTCTGGAAGAATTAATGTCAACTTTCT  
ATAGTTAGAAATAACTTAATTTTCAAATCCCTTTTTATCTTTGATTCACATGAATATCTAAGATTTGCTTTATAT  
TGCAAATTTCAAATTTGATGTTAAGTTATTAATAAAGTATCACATGAACAAGAGAGAGAGAAAGTAGTTTAT

TGTTTAGAAATTTATCATCCACATGCAACTATGTTAATACTAACATATTTGTTTAAGTTGTTGTAATAGGATATG  
ATAAACTTTTAAACAACAAATTAAGTTTAACTATTAACAAATGTGTTAAAAGTGTTA

Purple font: LbCas12a\_gHKT12.1; orange font: LbCas12a\_gHKT12.2; yellow highlighted font:  
modified PAM and LbCas12a gRNA core region to prevent recut after HDR editing; red font: N217D  
(AAT to GAT).

❖ **gRNA expression cassettes and donor sequences for SIEPSPS1 locus (shown in the Supplemental Figure 9)**

- Dual U6-crR1-2.23<sup>EPSPS1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATCAAGGAATAGTTGGATTCTTCTAATTCTACTAAGTGTAGATAATCGTT  
CCTTCTCGTGCCATTTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; blue font: LbCas12a\_gRNA1, 23nt; purple  
font: LbCas12a\_gRNA2, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- Dual U6-crR1-3.23<sup>EPSPS1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATCAAGGAATAGTTGGATTCTTCTAATTCTACTAAGTGTAGATAATCTCC  
ACATCTCCTAGAGCCATTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; blue font: LbCas12a\_gRNA1, 23nt; orange  
font: LbCas12a\_gRNA3, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- Dual U6-gR2-4.23<sup>EPSPS1</sup> expression cassette:

TGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAGAGTCGACATAGCGA  
TTGtAATTTCTACTAAGTGTAGATAATCGTTCTTCTCGTGCCATTAATTTCTACTAAGTGTAGATCTCCAAA  
GTCTCTGTCCCTCTTTTTT

Red font: AtU6 promoter; green font: LbCas12a scaffolds; purple font: LbCas12a\_gRNA2, 23nt; light  
blue font: LbCas12a\_gRNA4, 23nt; black G: transcription start; black TTTTTT: termination sequence.

- SIEPSPS1 donor 1-1 (EPSPS1D1) sequence

TGGTCCCCAAGTACTTAAATTGGTCCCCAAGTACTTTTCTGAGCTTAAGGTAATCAAACACTAATAAACATT  
TTCTTTGAGATCTGTCGTACCAAACATACTGAGTTTATATATTTGGTTCCAATGGTTTTGATACTTCATTTAAAT  
ACCATGTCACCTGCAATGAGCTTAAATATGCTTTTTACCACTTCAAGAAACATTGGTATTTAGCTGATTTTTGAA  
GTGCTACTTGCAATACTAATATATTAATATGTTAAAAGACATAAATTGTTTGACATCTTATTTTTGCTTTATG  
TTTTGTTTTTATTGAAATGATTGCATTTTGTCAATCTGTCTACCAAATTGATCTGCCAAATGTTCAACCTTTGTGA  
CATTTTGTGTTGGTTCTGGGGATTGAGCAATCCCTTTTTGGAGTTTCTGACCCTACCAGGTTGGCATTAGGGT  
GGGTTACTTATAAATTTGGACAATGGTTTTTTTTCTTAATTAATTCAAGGAGATATTTGTTAGAGTACCATCATT  
AATGATTTATGCAATCAACGCAAATTAAGTATATGGTAAAACTGATGCTATAAGTGTGAGGAGAGGAGCT  
AAGGGTTGAAAGAAACACTTAATTCTTAGTATAAGCTGTCCTAAACGACTGGGAATCTCTTCCAAGCTCAT  
GGTGTCTGAACAACACTCATACAGTATAATACACTCATAGCCATCGAGTGTGCAACCTTTTTAAGCTGACAAC  
TATTCAGTTCCATTGTTCTTTAACTTATCGTGTACATTTTCATGCTTTCATAATCTTGATATGAGTTACTTACAA  
CCTGTGCTGCCATTACATGTCACTGGCACTTGAATTTGAGGAAGGACTGTTGTTGACAATTTACTGAGTAGT



GACGACATTCATTACATGCTTGGTGC GTTAAAAACACTTGGACTTCATGTTGAAGATGACAATGAAAAACCAAC  
GAGCAATTGTGGAAGGTTGTGGTGGG CAGTTTCCTGTCGGTAAAAAGTCTGAGGAAGAAATCCAAC TATTCT  
TAGGTAAATGCA GCTACAGCAATGCGTCCGTTGACAGCAGCAGT TACTGTAGCTGGAGGACATTCAAGGTCTTT  
CAATCGTTCTTCTTCGTGCCATTTGTTTTGTCTT GTTTGTCTTTTTATTTTTCTCTTTTTTCCAGTGTGTTT  
CTTTGTCTTTTTTTTTTTTTTTGGGGGGGGGGGGTGTCTTTCTTTTGAAGCCATTTTGATAGTCTCATGAATTT  
GTATTTTTGGGAATGGTGGAAAAC TCTGAAATGTACATCGATAATTTAATTTTTTTTTGTTAGATAATAGTTG  
CCCTGGTGATAAATTACATGTCAGTTAGCTCCTCAAAGGTT CAGTAATCTGGATGTGGTGTCTTGGAGCTTC  
AGAAATGCATGCATACTTTGTACGTCATGTGTTTTACCATCTTTCTCAACGTGACAGAACAAGACAGGAAACG  
AGCATATTTTTGTTTGTCTTCTTTAGTTAGAGTTGTTTTCTTTCTTTTTTTGGTCTAGAAAATATTACCTTG  
TGGTGGGTGATAACTCTATGAATAATTGTGTGCTACAGATATGTTCTTGATGGAGTTCTAGGATGAGAGAA  
GACCAATTGGTGATTTGGTTGATGGTCTTAAGCAGCTTGGCGCAGAGGTAGATTGTTCCCTTGGTACGAATTG  
TCCCCAGTTCGAATTGTCAGCAAGGGAGG ACTTCCAGGAGGGAAGGTGAGGATTATGATTTTATTACGTAT  
AATGTTAGTGATTGGCAGCCTGTTACTGCAATTTTCTTAACTAGGAATAGTATTATCACTATTATTTAAAAAA  
AGAAAAAAGGCATAGTATTGTCATATGATGAGTAATTTTTAAATGCAATAGCATTTTTTCTTGAAAAGTTAA  
ACATACCTTAAACTTCGGAGAAAAC TGGCTTACATTATCTTATTTTAGATCCTGAGTAATAAAGCCAAAGATCCC  
TTCATCAGTTGGATACGATGAGTTATAGATGGAAATGAGTGGTGGT GAGACTTGAACCTGAAACCTCAACCT  
TGTTCTGGTACCATCTTGAATTATGTGTACAGCCTCTCTATAAAGCTTCAGCTATAAGAGAGGGCACACCTATA  
TTTCATTTGATTGTATTGTAACAAACATATTTTTAATCTCCATCTGATTTACCTTCATTTTATTTCATTTTATACT  
TTTTCTTAGAACTACTGACCTTAAAGTCTAAGAACAAGTATGCACTCACTCCCTCGTGCCATAGGTTAAATTAG  
TTTATAGTCAATATGTTTCTTGCATGTATCAAGCTCGTATGATAACAAGCTATTCAACTCTAGTATCTATCCAC  
TTAACGAGAAGTCTAAATGGGAAAGGCAGACTTCCAATTTTTCAAGTAACCTTGCTGTCCTCCTTATTTTGTCT  
TTTGTTCCTCCAAAAGTCTTGTGCCCTCCATTTGATCTCTCCACATTCCTTGTGCTGCCACTAAATTAAGA  
AGATGCATTTGATTCTGCAATATTATTTTATATTGATGAATCATGTT CATATGTTGTCTGTGGTTACTTGATCA  
GCTTCTTCTAGCATGGAAAGATGTAATATAACCGTTTTT GAGTTCTTGCTAAGTTATACTCCCTTCATATCTA  
TCAGGTAAAGCTCTCTGGATCCATCAGCAGCCAATACCTGACTGCTCTGCTTATGGCTGCTCCCTGACTCTAG  
GAGATGTGGAGATCGAGATAATTGACAAACTGATATCTGTGCCTTATGTTGAAATGCACTGAAGTTGATGG  
AGCGATTTGGTGTCTTTGTGGAGCACAGTAGTGGCTGGGACAGATTCTTGGTAAAAGGAGGTCAGAAGTACA  
AGTAAGTCGGCTTTCACACTGTTTTAATGTCTTTAATAATCTGGTTCCACCGATCAATGAAGCTGACAGTTACG  
AAAATATCATAGATCTCCTGGGAAAGCATTGTTGAAGGAGATGCCTCAAGTGCTAGCTATTTTTTGGCGGG  
GCAGCAGTCACAGGTGGAACCGTCACTGTTGAAGGTTGTGGAACAAGCAGTTTACAGGTATTTCTCATGTCAT  
TAAGACCTTGGGGCAATCACATTCTAGACTTTTCCATCTTGTGCTTGTATTTGTGACAGCGCCATAATACGTT  
CTATTTCTTCTGTTCACTAGCTTAATTTGTGGAAGTACTAAAAAGTAAGAATTGCACAGTGCATGGCCTACA  
TCTTAGTGTATAACTGCATAAGGTAATTTTCTAACAAGGGACTTACTTTGAGCTTTATGTTTCTTTGTGAAGG  
GAGATGTTAAGTTCGCTGAGGTCCTCGAGAAGATGGGGGCAGAAGTTACATGGACAGAGAACAGTGT CACA  
GTTAAAGGACCTCCGAGGAACTCTTCTGGAATGAAACATTTGCGTGCCATTGACGTGAACATGAACAAAATGC  
CAGATGTGGCCATGACTCTTGCCGTAGTTGCACTTTTTGTGATGGTCTACTACCATAAGAGATGGTATGGCT  
AACCTTGTTCCTGGTATCAAATCCTATTGAAGCACTTCGTTTAGATTAAGATATTATTCTGAAGCGATTGGG  
ACCTGGGGCTCTATTCTCCAATATGAAAGCATTTCCTGCATAATTAGTTTTTTTTAAATGCTGTGATAACCTGAC  
TAAATGTGCAAAGAGTTTTCTGGTAGTTTAGGGTCTG

Blue font: 2x TALE binding site (scTALE ABL1 R); yellow highlighted font: G177A; turquoise highlighted font: A268T; red fonts: base changes (without changing a.a coding) for modification of

gRNA binding sequences and PAM; orange fonts: red fonts: base changes (without changing a.a coding) for modification of Bpil, Bsal or BsmBI restriction sites for facilitating Golden gate cloning.

- SIEPSP1 donor 1-3 (EPSPS1D3) sequence

TTGGTCCCCAAGTACTTAAATTGGTCCCCAAGTACTTTTCCTGAGCTTAAGGTAATCAAACACTAATAAACATT  
TTCTTTGAGATCTGTCGTACCAAACATACTGAGTTTATATATTTGGTTCCAATGGTTTTGATACTTCATTTAAAT  
ACCATGTCACCTGCAATGAGCTTAAATATGCTTTTTACCACTTCAAGAAACATTGGTATTTAGCTGATTTTTGAA  
GTGCTACTTGCAATACTAATATATTAATATGTTAAAAGACATAAATTGTTTGCACATCTTATTTTTTGTCTTATG  
TTTTGTTTTTATTGAAATGATTGCATTTTGTCAATCTGTCTACCAAATTGATCTGCCAAATGTTCAACCTTTGTGA  
CATTTTGTGTGGTTCTGGGGATTGAGCAATCCCTTTTTGGAGTTTCTGACCCTACCAGGTTGGCATTAGGGT  
GGGTTACTTATAAATTTGGACAATGGTTTTTTTTCTTAATTAATTCAAGGAGATATTTGTTAGAGTACCATCATT  
AATGATTTATGCAAATCAACGCAAATTAAGTATATGGTAAAACTGATGCTATAAGTGTGAGGAGAGGAGCT  
AAGGGTTGAAAGAAACACTTAATTCTTAGTATAAGCTGTCCTAAACGACTGGGAACCTCTTCCCAAGCTCAT  
GGTGTCTGAACAACTCATACAGTATAATACACTCATAGCCATCGAGTGTGCAACCTTTTTAAGCTGACAACCTA  
TATTCAGTTCCATTGTTCTTTAACTTATCGTGTACATTTTCATGCTTTCATAATCTTGATATGAGTTACTTACAA  
CCTTGCTGCCATTACATGTCACTGGCACTTGAATTTAGGGAAGGACTGTTGTTGACAATTTACTGAGTAGT  
GACGACATTCATTACATGCTTGGTGC GTTAAAACTTGGACTTCATGTTGAAGATGACAATGAAAACCAAC  
GAGCAATTGTGGAAGGTTGTGGTGGGCAGTTTCTGTGCGTAAAAAGTCTGAGGAAGAAATCCAACCTATTCT  
TAGGTAAATGCAGGAATCGCAATGCGTTCTTTGACAGCAGCAGTACTGTAGCTGGAGGACATTCAAGGTCTAT  
CCATCGTTCCTTCTTCGTGCCATTTGTTTTGTCTTGGTTGCTTTTTATTTTTCTTCTTTTTTCCAGTGTTGTTT  
CTTTGTCTTTTTTTTTTTTTGGGGGGGGGGGGTGTCTTTTGAAGCCATTTGATAGTCTCATGAATTT  
GTATTTTTGGGAATGGTGGAAAACCTCTGAAATGTACATCGATAATTTAATTTTTTTTTGTTAGATAATAGTTG  
CCCTGGTGATAAATTACATGTCAGTTAGCTCCTCAAAGGTTCAAGTAATCTGGATGTGGTGCTTTGAGAGCTTC  
AGAAATGCATGCATACTTTGTACGTCATGTGTTTTACCATCTTCTCAACGTGACAGAACAAGACAGGAAACG  
AGCATATTTTTGTTTGTCTTCTTTAGTTAGAGTTGTTTTCTTTCTTTTTTTTTGGTCTAGAAAATATTACCTG  
TGGTGGGTGATAACTCTATGAATAATTGTGTGCTACAGATATGTTCTTGATGGAGTTCCTAGGATGAGAGAA  
GACCAATTGGTGATTTGGTTGATGGTCTTAAGCAGCTTGGCGCAGAGGTAGATTGTTCCCTTGGTACGAATTG  
TCCCCAGTTCGAATTGTCAGCAAGGGAGGACTTCCAGGAGGGAAGGTGAGGATTATGATTTTATTACGTAT  
AATGTTAGTGATTGGCAGCCTGTTACTGCAATTTCTTAAGTGAATAGTATTATCACTATTATTTAAAAAA  
AGAAAAAAGGCATAGTATTGTCACTATGATGAGTAATTTTTAAATGCAATAGCATTTTTTCTTGAAAAGTTAA  
ACATACCTTAACTTCGGAGAACTGGCTTACATTATCTTATTTTAGATCCTGAGTAATAAAGCCAAAGATCCC  
TCTCATCAGTTGGATACGATGAGTTATAGATGGAAATGAGTGGTGGTGAGACTTGAACCTGAAACCTCAACCT  
TGTTCTGGTACCATCTTGAATTATGTGTACAGCCTCTCTATAAAGCTTCAGCTATAAG

Blue font: 2x TALE binding site (scTALE ABL1 R); yellow highlighted font: T178I; turquoise highlighted font: P182S; red fonts: base changes (without changing a.a coding) for modification of gRNA binding sequences and PAM; orange fonts: red fonts: base changes (without changing a.a coding) for modification of Bpil, Bsal or BsmBI restriction sites for facilitating Golden gate cloning.

- SIEPSP1 donor 1-4 (EPSPS1D4) sequence

TTGGTCCCCAAGTACTTAAATTGGTCCCCAAGTACTTTTCTGAGCTTAAGGTAATCAAACACTAATAAACATT  
TTCTTTGAGATCTGTCGTACCAAACATACTGAGTTTATATATTTGGTTCCAATGGTTTTGATACTTCATTTAAAT  
ACCATGTCACCTGCAATGAGCTTAAATATGCTTTTTACCACTTCAAGAAACATTGGTATTTAGCTGATTTTTGAA  
GTGCTACTTGCAATACTAATATATTAATATGTTAAAAGACATAAATTGTTTGCACATCTTATTTTTGCTTTATG  
TTTTGTTTTTATTGAAATGATTGCATTTTGTCAATCTGTCTACCAAATTGATCTGCCAAATGTTCAACCTTTGTGA  
CATTTTGTGTGGTTCTGGGGATTGAGCAATCCCTTTTTGGAGTTTCTGACCCTACCAGGTTGGCATTAGGGT  
GGGTTACTTATAAATTTGGACAATGGTTTTTTTTCTTAATTAATTCAAGGAGATATTTGTTAGAGTACCATCATT  
AATGATTTATGCAATCAACGCAAATTAAGTATATGGTAAAACTGATGCTATAAGTGTGAGGAGAGGAGCT  
AAGGGTTGAAAGAAACACTTAATTCTTAGTATAAGCTGTCCTAAACGACTGGGAACTCTTCCCAAGCTCAT  
GGTGTCTGAACAACATACAGTATAATACACTCATAGCCATCGAGTGTGCAACCTTTTTAAGCTGACAACATA  
TATTCACGTTCCATTGTTCTTTAACTTATCGTGTACATTTTCATGCTTTCATAATCTTGATATGAGTTACTTACAA  
CCTTGCTGCCATTACATGTCACTGGCACTTGAATTTACAGGAAGGACTGTTGTTGACAATTTACTGAGTAGT  
GACGACATTCATTACATGCTTGGTGC GTTAAAACACTTGGACTTCATGTTGAAGATGACAATGAAAACCAAC  
GAGCAATTGTGGAAGGTTGTGGTGGGCAGTTTCTGTGCGTAAAAAGTCTGAGGAAGAAATCCAACATTTCT  
TAGGTAATGCAAGCTACAGCAATGCGTCCGTTGACAGCAGCAGTTACTGTAGCTGGAGGACATTCAAGGTCTG  
TCCATCGTTCCTTCTCGTGCCATTTGTTTTGTCTTGTTGCTTTTTATTTTTCTTCTTTTTTCCAGTGTGTT  
TCTTTGCTTTTTTTTTTTTTTTTTGGGGGGGGGGGGTGTCTGTTTCTTTGAAGCCATTTGATAGTCTCATGAATT  
TGTATTTTTGGGAATGGTGGAAAACCTCTGAAATGTACATCGATAATTAATTTTTTTTTGTTAGATAATAGTTG  
CCCTGGTGATAAATTACATGTCAAGTTAGCTCCTCAAAGTTCAGTAATCTGGATGTGGTGCTTTGAGAGCTTC  
AGAAATGCATGCATACTTTGTACGTCATGTGTTTTACCATCTTCTCAACGTGACAGAACAAGACAGGAAACG  
AGCATATTTTTGTTGCTTCTTCTTTAGTTAGAGTTGTTTTCTTTCTTTTTTTTTGGTCTAGAAAATATTACCTG  
TGGTGGGTGATAACTCTATGAATAATTGTGTGCTACAGATATGTTCTTGATGGAGTTCTAGGATGAGAGAA  
GACCAATTGGTGATTTGGTTGATGGTCTTAAGCAGCTTGGCGCAGAGGTAGATTGTTCCCTTGGTACGAATTG  
TCCCCAGTTGCAATTGTCAGCAAGGGAGGACTTCCAGGAGGGAAGGTGAGGATTATGATTTTATTACGTAT  
AATGTTAGTGATTGGCAGCCTGTTACTGCAATTTCTTAAGTGAATAGTATTATCACTATTATTTAAAAAA  
AGAAAAAAGGCATAGTATTGTCACTATGATGAGTAATTTTTAAATGCAATAGCATTTTTTCTTGAAAAGTTAA  
ACATACCTTAACTTCGGAGAACTGGCTTACATTATCTTATTTTAGATCCTGAGTAATAAAGCCAAAGATCCC  
TCTCATCAGTTGGATACGATGAGTTATAGATGAAATGAGTGGTGGTGGAGACTTGAACCTGAAACCTCAACCT  
TGTTCTGGTACCATCTTGAATTATGTGTACAGCCTCTCTATAAAGCTTACAGCTATAAGAGAGGGCACACCTATA  
TTTCATTTGATTGTATTGTAACAAACATATTTTTAATCTCCATCTGATTTACCTTCATTTTATTTTACT  
TTTTCTTAGAACTACTGACCTTAAAGTCTAAGAACAAGTATGCACTCACTCCCTCGTGCCATAGGTTAAATTAG  
TTTATAGTCAATATGTTTCTTGCATGTATCAAGCTCGTATGATAACAAGCTATTCAACTCTAGTATCTATCCAC  
TTAACGAGAAGTCTAAATGGGAAAGGCAGACTTCCAATTTTTCAAGTAACCTTGGTGTCTCCTTTATTTTGTCT  
TTTGTATCATCCAAAAGTCTCTTGTCCCTCCATTTGATCTCTCCACATTCCTTGTGCTGCCACTAAATTAAGA  
AGATGCATTTGATTCTGCAATATTATTTTATATTGATGAATCATGTTTCATATGTTGTCTGTGGTTTACTTGATCA  
GCTTCTTCTAGCATGGAAAGATGTAATATAACCGTTTTTGGATTCTTGTGTAAGTTATACTCCCTTCATATCTA  
TCAGGTAAAGCTCTCTGGATCCATCAGCAGCCAATACCTGACTGCTCTGCTTATGGCTGCTCCCCTGACTCTAG  
GAGATGTGGAGATCGAGATAATTGACAACTGATATCTGTGCCTTATGTTGAAATGCACTGAAGTTGATGG  
AGCGATTTGGTGTCTTTGTGGAGCACAGTAGTGGCTGGGACAGATTCTTGGTAAAAGGAGGTCAGAAGTACA  
AGTAAGTCGGCTTTCACACTGTTTTAATGTCTTTAATAATCTGGTTCCACCGATCAATGAAGCTGACAGTTACG  
AAAATATCATAGATCTCCTGGGAAAGCATTTGTTGAAGGAGATGCCTCAAGTGCTAGCTATTTTTTGGCGGGG  
GCAGCAGTCACAGGTGGAACCGTCACTGTTGAAGGTTGTGGAACAAGCAGTTTACAGGTATTTCTCATGTCAT

TAAGACCTTGGGGGCAATCACATTCTAGACTTTTCATCTTGTGCTTGTATTTGTGACAGCGCCATAATACGTT  
CTATTTCTTCTGTTCACTAGCTTAATTTGTGGAAGTACTAAAAAGTAAGAATTGCACAGTGCATGGCCTACA  
TCTTAGTGTATAACTGCATAAGGTAATTTCTACAAGGGACTTACTTTGAGCTTTATGTTTCCTTTGTGAAGG  
GAGATGTTAAGTTCGCTGAGGTCCTCGAGAAGATGGGGGCAGAAGTTACATGGACAGAGAACAGTGTCA  
GTTAAAGGACCTCCGAGGAACTCTTCTGGAATGAAACATTTGCGTGCCATTGACGTGAACATGAACAAAATGC  
CAGATGTGGCCATGACTCTTGCCGTAGTTGCACTTTTTGCTGATGGTCCCTACTACCATAAGAGATGGTATGGCT  
AACCTGTTTCCCTGGTATCAAATCCTATTGAAGCACTTCGTTTAGATTAAGATATTATTCTGAAGCGATTGGG  
ACCTGGGGCTCTATTCTCCAATATGAAAGCATTCCCTGCATAATTAGTTTTTTAAATGCTGTGATAACCTGAC  
TAAATGTGCAAAGAGGTTTTCTGGTAGTTTAGGGTCTG

Blue font: 2x TALE binding site (scTALE ABL1 R); yellow highlighted font: G177A; turquoise highlighted font: A268T; red fonts: base changes (without changing a.a coding) for modification of gRNA binding sequences and PAM; orange fonts: red fonts: base changes (without changing a.a coding) for modification of Bpil, Bsal or BsmBI restriction sites for facilitating Golden gate cloning.

❖ **LbCas12a (wild-type) expression cassette:**

GAATTCCAATCCACAAAAATCTGAGCTTAACAGCACAGTTGCTCCTCTCAGAGCAGAATCGGGTATTCAACACCC  
TCATATCAACTACTACGTTGTGTATAACGGTCCACATGCCGTATATACGATGACTGGGGTTGTACAAAGGCGGCA  
ACAAACGGCGTCCCGGAGTTGCACACAAGAAATTTGCCACTATTACAGAGGCAAGAGCAGCAGCTGACGCGTAC  
ACAACAAGTCAGCAAACAGACAGGTTGAACTTCATCCCAAAGGAGAAGCTCAACTCAAGCCCAAGAGCTTTGCT  
AAGGCCCTAAACAAGCCCACCAAAGCAAAAAGCCACTGGCTCACGCTAGGAACCAAAGGCCAGCAGTGATCCA  
GCCCCAAAAGAGATCTCCTTTGCCCGGAGATTACAATGGACGATTTCTCTATCTTTACGATCTAGGAAGGAAGT  
TCGAAGGTGAAGGTGACGACACTATGTTCACTGATAATGAGAAGGTTAGCCTCTTCAATTTAGAAAGAATGC  
TGACCCACAGATGGTTAGAGAGGCCTACGACGCAAGTCTCATCAAGACGATCTACCCGAGTAACAATCTCCAGGA  
GATCAAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAAGATTCAGGACTAATTGCATCAAGAACACAGAGAA  
AGACATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCATAAACAAGGCAAGTA  
ATAGAGATTGGAGTCTCTAAAAAGGTAGTTCTACTGAATCTAAGGCCATGCATGGAGTCTAAGATTCAAATCGAG  
GATCTAACAGAACTCGCCGTCAGACTGGCGAACAGTTTACATACAGAGTCTTTTACGACTCAATGACAAGAAGAAA  
ATCTTCGTCAACATGGTGGAGCACGACACTCTGGTCTACTCCAAAAATGTCAAAGATACAGTCTCAGAAGATCAAA  
GGGCTATTGAGACTTTTCAACAAAAGGATAATTCGGGAAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTC  
ATCGAAAGGACAGTAGAAAAGGAAGGTGGCTCTACAAATGCCATCATTGCGATAAAGGAAAGGCTATCATTCAA  
GATCTCTGCCCACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAAGGTTCCA  
ACCACGTCTACAAAGCAAGTGGATTGATGTGACATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTT  
CGCAAGACCCCTTCTATATAAGGAAGTTCATTTCAATTTGGAGAGGACACGCTCGAGTATAAGGTAAATTTCTGT  
GTTCTTATTCTCTCAAAATCTTCGATTTTGTTCGTTTCGATCCCAATTCGTATATGTTCTTTGGTTTAGATTCTGTT  
AATCTTAGATCGAAGATGATTTTCTGGGTTTGATCGTTAGATATCATCTTAATTCTCGATTAGGGTTTCATAGATATC  
ATCCGATTTGTTCAATAATTTGAGTTTTGTGCAATAACTCTTCGATTTGTGATTTCTATCTAGATCTGGTGTTA  
GTTTCTAGTTTGTGCGATCGAATTTGTGATTAATCTGAGTTTTTCTGATTAACAGGAGCTATTTTACAACAATTA  
CCAACAACAACAACAACAACAACATTACAATTACATTTACAATTATCGATACAATGATGCCAAGAAGAAGCGC  
AAGGTGGGACGCGTCTGCAGGATATCAAGCTTGGGTACCGCGGGCCCGGATCGCCACCATGAGCAAGCTGGA  
GAAGTTTCAAACTGCTACTCCCTGTCTAAGACCCTGAGGTTCAAGGTAAGCCTCGATTTTTGGGTTTAGGTGTCT

GCTTATTAGAGTAAAAACACATCCTTTGAAATTGTTTGTGGTCATTTGATTGTGCTCTTGATCCATTGAATTGCTGCA  
GGCCATCCCTGTGGGCAAGACCCAGGAGAACATCGACAATAAGCGGCTGCTGGTGGAGGACGAGAAGAGAGCC  
GAGGATTATAAGGGCGTGAAGAAGCTGCTGGATCGCTACTATCTGTCTTTATCAACGACGTGCTGCACAGCATCA  
AGCTGAAGAATCTGAACAATTACATCAGCCTGTTCCGGAAGAAAACCAGAACCGAGAAGGAGAATAAGGAGCTG  
GAGAACCTGGAGATCAATCTGCGGAAGGAGATCGCCAAGGCCCTCAAGGGCAACGAGGGCTACAAGTCCCTGTT  
TAAGAAGGATATCATCGAGACAATCCTGCCAGAGTTCCTGGACGATAAGGACGAGATCGCCCTGGTGAACAGCTT  
CAATGGCTTTACCACAGCCTTACC CGCTTCTTTGATAACAGAGAGAATATGTTTTCCGAGGAGGCCAAGAGCACA  
TCCATCGCCTTCAGGTGTATCAACGAGAATCTGACCCGCTACATCTCTAATATGGACATCTTCGAGAAGGTGGACG  
CCATCTTTGATAAGCACGAGGTGCAGGAGATCAAGGAGAAGATCCTGAACAGCGACTATGATGTGGAGGATTTCT  
TTGAGGGCGAGTCTTTAACTTTGTGCTGACACAGGAGGGCATCGACGTGTATAACGCCATCATCGGCGGCTTCGT  
GACCGAGAGCGGCGAGAAGATCAAGGGCCTGAACGAGTACATCAACCTGTATAATCAGAAAACCAAGCAGAAGC  
TGCCTAAGTTAAGCCACTGTATAAGCAGGTGCTGAGCGATCGGGAGTCTCTGAGCTTCTACGGCGAGGGCTATA  
CATCCGATGAGGAGGTGCTGGAGGTGTTTAGAAACACCCTGAACAAGAACAGCGAGATCTTCAGCTCCATCAAGA  
AGCTGGAGAAGCTGTTCAAGAATTTGACGAGTACTCTAGCGCCGGCATCTTTGTGAAGAACGGCCCCGCCATCA  
GCACAATCTCCAAGGATATCTTCGGCGAGTGGAACGTGATCCGGGACAAGTGAATGCCGAGTATGACGATATCC  
ACCTGAAGAAGAAGGCCGTGGTGACCGAGAAGTACGAGGACGATCGGAGAAAGTCCTTCAAGAAGATCGGCTCC  
TTTTCTCTGGAGCAGCTGCAGGAGTACGCCGACGCCGATCTGTCTGTGGTGGAGAAGCTGAAGGAGATCATCATC  
CAGAAGGTGGATGAGATCTACAAGGTGTATGGCTCCTCTGAGAAGCTGTTCCGACGCCGATTTTGTGCTGGAGAAG  
AGCCTGAAGAAGAACGACGCCGTGGTGGCCATCATGAAGGACCTGCTGGATTCTGTGAAGAGCTTCGAGAATTAC  
ATCAAGGCCTTCTTTGGCGAGGGCAAGGAGACAAACAGGGACGAGTCTTCTATGGCGATTTTGTGCTGGCCTAC  
GACATCCTGCTGAAGGTGGACCACATCTACGATGCCATCCGCAATTATGTGACCCAGAAGCCCTACTCTAAGGATA  
AGTTCAAGCTGTATTTTCAGAACCCTCAGTTCATGGGCGGCTGGGACAAGGATAAAGGAGACAGACTATCGGGCCA  
CCATCCTGAGATACGGCTCCAAGTACTATCTGGCCATCATGGATAAGAAGTACGCCAAGTGCCTGCAGAAGATCG  
ACAAGGACGATGTGAACGGCAATTACGAGAAGATCAACTATAAGCTGCTGCCCGGCCCTAATAAGATGCTGCCAA  
AGGTGTTCTTTTCTAAGAAGTGGATGGCCTACTATAACCCAGCGAGGACATCCAGAAGATCTACAAGAATGGCA  
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AGAGAGGTGGAGGAGCAGGGCTATAAGGTGAGCTTCGAGTCTGCCAGCAAGAAGGAGGTGGATAAGCTGGTGG  
AGGAGGGCAAGCTGTATATGTTCCAGATCTATAACAAGGACTTTTCCGATAAGTCTCACGGCACACCCAATCTGCA  
CACCATGTACTTCAAGCTGCTGTTTGACGAGAACAATCACGGACAGATCAGGCTGAGCGGAGGAGCAGAGCTGTT  
CATGAGGCGCGCCTCCCTGAAGAAGGAGGAGCTGGTGGTGACCCAGCCAACCTCCCTATCGCCAACAAGAATCC  
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GGCCTTCTACTCTAGCTTTATGGCCCTGATGAGCCTGATGCTGCAGATGCGGAACAGCATCACAGGCCGCACCGAC  
GTGGATTTTCTGATCAGCCCTGTGAAGAACTCCGACGGCATCTTCTACGATAGCCGAACTATGAGGCCAGGAG  
AATGCCATCCTGCCAAGAAGCGCCGACGCCAATGGCGCCTATAACATCGCCAGAAAGGTGCTGTGGGCCATCGGC  
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CGCCAGACCAGCGTGAAGCACGCCTATCCCTATGACGTGCCGATTATGCCAGCCTGGGCAGCGGCTCCCCAA  
GAAAAACGCAAGGTGGAAGATCCTAAGAAAAAGCGGAAAGTGGACGGCATTGGTAGTGGGAGCTAAGCTTCTC  
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CCTATAGGGTTTCGCTCATGTGTTGAGCATATAAGAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAA  
TAAAATTTCTAATTCCTAAAACCAAATCCAGTACTAAAATCCAGATC

Purple font: SV40 NLS; blue font: LbCas12a; green font: HA tag; orange font: CaMV 35S promoter; tan font: Trp1 intron; dark blue: AtUBQ10 intron 1; black font: linkers.

❖ **ttLbCas12a (temperature-tolerant) expression cassette:**

GAATTCGAATCCCAAAAAATCTGAGCTTAACAGCACAGTTGCTCCTCTCAGAGCAGAATCGGGTATTCAACACCC  
TCATATCAACTACTACGTTGTGTATAACGGTCCACATGCCGGTATATACGATGACTGGGGTTGTACAAAGGCGGCA  
ACAAACGGCGTTCCCGGAGTTGCACACAAGAAATTTGCCACTATTACAGAGGCAAGAGCAGCAGCTGACGCGTAC  
ACAACAAGTCAGCAAACAGACAGGTTGAACTTCATCCCAAAGGAGAAGCTCAACTCAAGCCCAAGAGCTTTGCT  
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GCCCCAAAAGAGATCTCCTTTGCCCGGAGATTACAATGGACGATTTCTCTATCTTTACGATCTAGGAAGGAAGT  
TCGAAGGTGAAGGTGACGACACTATGTTCAACTGATAATGAGAAGGTTAGCCTCTTCAATTTAGAAAGAATGC  
TGACCCACAGATGGTTAGAGAGGCCTACGACGCAAGTCTCATCAAGACGATCTACCCGAGTAACAATCTCCAGGA  
GATCAAATACCTTCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAATTGCATCAAGAACACAGAGAA  
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ATAGAGATTGGAGTCTCTAAAAAGGTAGTTCTACTGAATCTAAGGCCATGCATGGAGTCTAAGATTCAAATCGAG  
GATTAACAGAACTCGCCGTCAGACTGGCGAACAGTTTCATACAGAGTCTTTTACGACTCAATGACAAGAAGAAA  
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CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGGACACGCTCGAGTATAAGGTAATTTCTGT  
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CCAACAACAACAACAACAACAACATTACAATTACATTTACAATTATCGATACAATGATGCCAAGAAGAAGCGC  
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CACCATGTACTTCAAGCTGCTGTTTACGAGAACAATCACGGACAGATCAGGCTGAGCGGAGGAGCAGAGCTGTT  
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GATTACCACTCTCTGCTGGACAAGAAGGAGAAGGAGAGGTTTCGAGGCCCGCCAGAAGTGGACCTCCATCGAGAA  
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CATCCCTGCCTGGCTGACATCAAGATCGATCCATCTACCGGCTTTGTGAACCTGCTGAAAACCAAGTATAACCAGC  
ATCGCCGATTCAAGAAGTTCATCAGCTCCTTTGACAGGATCATGTACGTGCCGAGGAGGATCTGTTTCGAGTTT

CCCTGGACTATAAGAACTTCTCTCGCACAGACGCCGATTACATCAAGAAGTGGAAGCTGTACTCCTACGGCAACCG  
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GGCCTTCTACTCTAGCTTTATGGCCCTGATGAGCCTGATGCTGCAGATGCGGAACAGCATCACAGGCCGCACCGAC  
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CCTATAGGGTTTCGCTCATGTGTTGAGCATATAAGAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAA  
TAAAATTTCTAATTCCTAAAACCAAATCCAGTACTAAAATCCAGATC

Purple font: SV40 NLS; blue font: LbCas12a; green font: HA tag; orange font: CaMV 35S promoter; tan  
font: Trp1 intron; dark blue: AtUBQ10 intron 1; yellow highlighted font: D156R modification of the  
temperature tolerant LbCas12a; black font: linkers.

- ❖ **SpCas9 expression cassette:** pNOS-NptII-tOCS cloned from plasmid pICH47742::2x35S-5'UTR-hCas9(STOP)-NOST (Addgene Plasmid #49771).
- ❖ **Plant selection marker:** pNOS-NptII-tOCS cloned from pICSL11024 (pICH47732::NOSp-NPTII-OCST) (Addgene Plasmid #51144).