Supplementary Material

# Supplementary Figures and Tables

## Supplementary Figures

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| **Supplementary Figure 1**. Construction of (A) the Cas9 expression vector and (B) the Cas9-GFP expression vector. |

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| **Supplementary Figure 2**. Bioinformatics workflow to extract genome-edited alleles from HiPlex amplicon sequencing data |

## Supplementary Tables

Supplementary Table 1. Oligonucleotide and primer sequences. Underlined = N20 guide RNA sequence, *Italic* = vector overhangs

|  |  |  |
| --- | --- | --- |
| **Oligo/Primer** | **Sequence** | **Purpose** |
| Oligo1 | 5’-*ATTG*TTTGGATGCTGTGATGCCAT-3’ | PDS oligo F |
| Oligo2 | 5’-*AAAC*ATGGCATCACAGCATCCAAA-3’ | PDS oligo R |
| Oligo3 | 5’-*ATTG*CACATGGCAGTTTGTGACCA-3’ | GAS oligo F |
| Oligo4 | 5’-*AAAC*TGGTCACAAACTGCCATGTG-3’ | GAS oligo R |
| Oligo5 | 5’-*ATTG*AGATTGTTAAAGAGATCTTG-3’ | GAO oligo F |
| Oligo6 | 5’-*AAAC*CAAGATCTCTTTAACAATCT-3’ | GAO oligo R |
| Oligo7 | 5’-*ATTG*TTTCAGCTCCATTCGCGAAG-3’ | COS oligo F |
| Oligo8 | 5’-*AAAC*CTTCGCGAATGGAGCTGAAA-3’ | COS oligo R |
| Oligo9 | 5’-*ATTG*GGAGACCCGAGGGTCTCT-3’ | BsaI insert |
| Oligo10 | 5’-*AAAC*AGAGACCCTCGGGTCTCC-3’ | BsaI insert |
| Primer20 | 5’-TCCCAGGATTAGAATGATTAGG-3’ | pCas9\_F |
| Primer24 | 5’-CCAGGAAACAGCTATGACCAT-3’ | pCas9\_R |
| BbsIApaI\_S | 5’-GGCCCAGTCATCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCTACTCTCGCGTTAACGCTAGCATGGATGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTCTTAAGCTCGGGCC-3’ | BbsI mutated insert |
| Bbs1Apa1\_AS | 5’-CGAGCTTAAGACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACATCCATGCTAGCGTTAACGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGATGACTG-3’ | BbsI mutated insert |

Supplementary Table 2. Vector overview. aThe cloning sites are four-nucleotides overhangs for Golden Gate cloning (according to Lampropoulos et al. 2013)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Full Name** | **Type** | **Cloning sitesa** | **Bacterial Selection** | **Length** | **Reference** | **Plasmid Deposit** |
| **Golden gate plant promotor** |  |  |  |  |  |  |
| pGGA004 | 35S (Cauliflower mosaic virus 35S) promoter | A-B | Ampicillin | 3550 bp | Lampropoulos et al. (2013) | Addgene ID 48814 |
|  |  |  |  |  |  |  |
| **Golden gate N-tags** |  |  |  |  |  |  |
| pGGB003 | B-dummy (default random sequence if no specific N-tag is desired) | B-C | Ampicillin | 2710 bp | Lampropoulos et al. (2013) | Addgene ID 48821 |
|  |  |  |  |  |  |  |
| **Golden gate C-tags** |  |  |  |  |  |  |
| pGGD001 | linker-GFP | D-E | Ampicillin | 3507 bp | Lampropoulos et al. (2013) | Addgene ID 48833 |
| pGGD002 | D-dummy (default random sequence with stop codon if no specific C-tag is desired) | D-E | Ampicillin | 2732 bp | Lampropoulos et al. (2013) | Addgene ID 48834 |
|  |  |  |  |  |  |  |
| **Golden gate plant terminator** |  |  |  |  |  |  |
| pGGE001 | RBCS terminator (from pea) | E-F | Ampicillin | 3333 bp | Lampropoulos et al. (2013) | Addgene ID 48839 |
|  |  |  |  |  |  |  |
| **Coding sequences** |  |  |  |  |  |  |
| pGG-C-Cas9PTA\*-D | *Cas9-SV40* with stop codon | C-D | Ampicillin | 6828 bp | Houbaert et al. (2018) |  |
| pGG-C-Cas9PTA-D | *Cas9-SV40* | C-D | Ampicillin | 6825 bp | (Decaestecker et al., 2019) |  |
|  |  |  |  |  |  |  |
| **Unarmed gRNA entry vectors** |  |  |  |  | Decaestecker et al. (2019) |  |
| pGG-F-AtU6-26-BbsI-BbsI-G | AtU6-26 promoter and 'unarmed' gRNA scaffold | F-G | Ampicillin | 3177 bp | Decaestecker et al. (2019) |  |
| pGG-F-AtU6-26-AarI-AarI-G | AtU6-26 promoter and 'unarmed' gRNA scaffold | F-G | Ampicillin | 3183 bp | Decaestecker et al. 2019 |  |
|  |  |  |  |  |  |  |
| **Destination vector** |  |  |  |  |  |  |
| pEN-L1-AG-L2 |  | A-G | Kanamycin | 3990 bp | Houbaert et al. (2018) |  |
| pEN-L1-AG-L2,\(-Bbs1) |  | A-G | Kanamycin | 3990 bp | This work |  |
|  |  |  |  |  |  |  |
| **Unarmed gRNA destination vectors** |  |  |  |  |  |  |
| pCDB-Cas9 |  |  | Kanamycin | 8789 bp | This work |  |
| pCDB-Cas9-GFP |  |  | Kanamycin | 9567 bp | This work |  |
|  |  |  |  |  |  |  |
| **Unarmed gRNA destination vectors** (ccdB+) |  |  |  |  |  |  |
| pCDB-Cas9-ccdB |  |  | Kanamycin | 10206 bp | This work |  |
| pCDB-Cas9-GFP-ccdB |  |  | Kanamycin | 10978 bp | This work |  |
|  |  |  |  |  |  |  |
| **Expression vectors** |  |  |  |  |  |  |
| pCDB-Cas9-PDS |  |  | Kanamycin | 8791 bp | This work |  |
| pCDB-Cas9-GFP-PDS |  |  | Kanamycin | 9559 bp | This work |  |
| pCDB-Cas9-GAS |  |  | Kanamycin | 8791 bp | This work |  |
| pCDB-Cas9-GAO |  |  | Kanamycin | 8791 bp | This work |  |
| pCDB-Cas9-COS |  |  | Kanamycin | 8791 bp | This work |  |
|  |  |  |  |  |  |  |
| **Inserts** |  |  |  |  |  |  |
| ccdB insert | ccdB gene |  |  |  | Decaestecker et al. (2019) |  |
|  |  |  |  |  |  |  |
| **Other** |  |  |  |  |  |  |
| pKAR6 | GFP vector without NLS signal |  | Carbenicillin | 4450 bp | Robert Blanvillain, unpublished data |  |

Supplementary Table 3. Primer sequences used for HiPlex amplicon construction and ddPCR.

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| --- | --- | --- | --- |
| **Primer** | **Sequence** | **Gene** | **Application** |
| Primer1 | 5‘-TTCAAGCACACAGATCACTTCA-3’ | PDS\_F | HiPlex |
| Primer2 | 5’-TGGTGTAAAAGAACGGGCACT-3’ | PDS\_R | HiPlex |
| Primer3 | 5’-GATGAGTTTGATCTATACACAACTTCT-3’ | GAS\_F | HiPlex |
| Primer4 | 5’-ATGGTGTGTCATGGTTGATCA-3’ | GAS\_R | HiPlex |
| Primer5 | 5’-ATGATCGCCACCATATTGAGCA-3’ | GAO\_F | HiPlex |
| Primer6 | 5’-TTGAAGGAAAGATATCAGCTACATCG-3’ | GAO\_R | HiPlex |
| Primer7 | 5’-GGCGAATACTGGAGGCAGAT-3’ | COS\_F | HiPlex |
| Primer8 | 5’-AGTTGATGGGTGTTCCTGCT-3’ | COS\_R | HiPlex |
| Primer 9 | 5’- TGCTTACCCTAGTGCCTCTGA-3’ | PP2AA3\_F | ddPCR |
| Primer 10 | 5’- TTCCCAAATTTGTAGCAGCA-3’ | PP2AA3\_R | ddPCR |
| Primer 11 | 5’- CCGCCATTAACTCAAGTGGA-3’ | PDS\_F | ddPCR |
| Primer 12 | 5’- TTGGGAATTGCAATCTGTGA-3’ | PDS\_R | ddPCR |

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| **Supplementary Table 4**. Two HiPlex detected *CiPDS* loci in all screened regenerated (control and albino) plants. Purple = PAM site, green = gRNA target site, dashed vertical line = cut site, blue = SNP. |
| |  |  | | --- | --- | | ***CiPDS*** | | | Locus 1 | AGACTGTTTCAACATCACCTCAGGACAAAAAGATGTACTCTCATTTGGATGCTGTGATGCCATGGGTCACAGATTGCAATTC | | Locus 2 | AGATTGTTTCAACATCACCTCAGGACAAAAAGATGTACTCTCATTTGGATGCTGTGATGCCATGGGTCACAGATTGCAATTC | |

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| **Supplementary Table 5**. Raw droplet digital PCR (ddPCR) data to quantify the copy number of *CiPDS* in a diploid ‘Van Hamme’ witloof plant. \*, Deviating droplet profile; sample was excluded from the analysis. |
| |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | | **#** | **Sample ID** | **Target** | **Positive droplets** | **Negative droplets** | **Total droplets** | **Concentration (copies.µL-1)** | | 1 | NTC | *CiPDS* | 0 | 17558 | 17558 | 0 | | 2 | NTC | *CiPDS* | 0 | 16678 | 16678 | 0 | | 3 | Van Hamme 1 | *CiPDS* | 11696 | 5140 | 16836 | 1396 | | 4 | Van Hamme 1 | *CiPDS* | 12272 | 5465 | 17737 | 1385 | | 5 | Van Hamme 2 | *CiPDS* | 12016 | 5812 | 17828 | 1319 | | 6 | Van Hamme 2 | *CiPDS* | 11607 | 5348 | 16955 | 1357 | | 7 | Van Hamme 3 | *CiPDS* | 11409 | 6053 | 17462 | 1246 | | 8 | Van Hamme 3 | *CiPDS* | 11001 | 5683 | 16684 | 1267 | | 9 | Van Hamme 4 | *CiPDS* | 9721 | 7490 | 17211 | 979 | | 10 | Van Hamme 4 | *CiPDS* | 9584 | 7303 | 16887 | 986 | | 11 | NTC | *PP2AA3* | 0 | 18601 | 18601 | 0 | | 12 | NTC | *PP2AA3* | 0 | 18648 | 18648 | 0 | | 13 | Van Hamme 1 | *PP2AA3* | 4805 | 15194 | 19999 | 323 | | 14 | Van Hamme 1 | *PP2AA3* | 4013 | 13268 | 17281 | 311 | | 15 | Van Hamme 2 | *PP2AA3* | 4015 | 13693 | 17708 | 303 | | 16 | Van Hamme 2 | *PP2AA3* | 2313\* | 11972\* | 14285\* | 208\* | | 17 | Van Hamme 3 | *PP2AA3* | 4126 | 14061 | 18187 | 303 | | 18 | Van Hamme 3 | *PP2AA3* | 3776 | 14027 | 17803 | 280 | | 19 | Van Hamme 4 | *PP2AA3* | 2793 | 14763 | 17556 | 204 | | 20 | Van Hamme 4 | *PP2AA3* | 3063 | 15287 | 18350 | 215 | | 21 | NTC | *UBQ10* | 0 | 17651 | 17651 | 0 | | 22 | NTC | *UBQ10* | 1 | 16842 | 16843 | 0.07 | | 23 | Van Hamme 1 | *UBQ10* | 6812 | 10860 | 17672 | 573 | | 24 | Van Hamme 1 | *UBQ10* | 6680 | 10226 | 16906 | 591 | | 25 | Van Hamme 2 | *UBQ10* | 6841 | 10549 | 17390 | 588 | | 26 | Van Hamme 2 | *UBQ10* | 6342 | 9603 | 15945 | 597 | | 27 | Van Hamme 3 | *UBQ10* | 5768 | 10663 | 16431 | 509 | | 28 | Van Hamme 3 | *UBQ10* | 5954 | 10103 | 16057 | 545 | | 29 | Van Hamme 4 | *UBQ10* | 4820 | 10753 | 15573 | 436 | | 30 | Van Hamme 4 | *UBQ10* | 4851 | 10693 | 15544 | 440 | |