

Supplementary material

S1. Sequence of the cDNA of the gene of interest with the sgRNA target sequences depicted.

S2. Sequence of sgRNA used and the primers.

sgRNA					
Name	Position	Strand	Sequence	PAM	Efficiency Score
sgRNA1	114	1	ATTAAAAGTAGAAAAAAGGG	TGG	54.67
sgRNA3	289	-1	GCAACAACAGCTCCAACAAT	AGG	42.47
sgRNA4	498	1	TCGTTTCTGCTCTTGTCAAT	TGG	27.15
sgRNA5	896	1	TTCAGGACCTCAAGTGCCAA	CGG	43.81
Primers used for IDAA					
Name	Type	Sequence		Concentration	
FAM	Universal FAM	5'-6-FAM-AGC TGA CCG GCA GCA AAA TTG-3'		0.25 µM	
Trans22_F_1	For-extension forward	5' AGC TGA CCG GCA GCA AAA TTG GCA AGA GAT GGC GGA GTA TC 3'		0.025 µM	
Trans22_R_2	Reverse	5' TGA AGA TGG AAC CAA GGC CA 3'		0.25 µM	

S3. Alignment of the mutated alleles with coding sequences (**A**) and predicted amino acid sequences (**B**). Red amino acids indicate change in reading frame.

S4. Media and Stock solutions recipes modified from Nicolia et al.(2015)

Medium PPM	1 liter
MS Salts + vitamins	4.3 g
Sucrose	20 g
Kinetin (10 mM stock)	100 µl
Agar	3.5 g
pH	5.8

Medium B	1 liter
MS modif. No 4	2.7 g
Vitamins NN stock	0.1 ml
Casein hydrolysate	100 mg
NAA Soluble in EtOH	2 mg
BAP Soluble in MeOH	0.5 mg
pH	5.8

Medium C	500 ml
Macro stock	5 ml
CaCl ₂ stock (2 M)	1.5 ml
Iron stock	5 ml
Micro stock	500 µl
Vit mix 1 stock	2.5 ml
Vit mix 2 stock	2.5 ml
Vit mix 3 stock	2.5 ml
Sugars stock	10 ml
Organic acids stock	5 ml
Casein hydrolysate	250 mg
Glucose 0.2 M	18.5 g
Mannitol 0.2M	18.5 g
PVP 10	10 g
NAA Soluble in EtOH	0.5 mg
BAP Soluble in MeOH	0.2 mg
Cellulase RS	5 g
Macerozyme	1.5 g
pH	5.6

Medium E	1 liter
Macro stock	10 ml
CaCl ₂ stock (2 M)	1.25 ml
Iron stock	10 ml
Micro stock	1 ml
Vit. Mix 1 stock	5 ml
Vit. Mix 2 stock	5 ml
Vit. Mix 3 stock	5 ml
Sugars stock	20 ml
Organic acids stock	10 ml

Casein hydrolysate	500 mg
Glucose	33,7 g
Mannitol	30,92 g
BSA	1 g
NAA	1 mg
BAP	0.4 mg
pH	5.6

Medium F	1 liter
MS modif. No 4	2,70 g
NH ₄ Cl	107 mg
Vit. NN stock	1 ml
Adenine sulphate	40 mg
Casein hydrolysate	100 mg
Sucrose	2.5 g
Mannitol	54,7 g
NAA	0.1 mg
BAP	0.5 mg
pH	5.8

Medium G	1 liter
MS modif. No 4	2,70 g
NH ₄ Cl	267.5 mg
Vit. NN stock	1 ml
Adenine (hemi)sulphate	80 mg
Casein hydrolysate	100 mg
Sucrose	2.5 g
Mannitol	36,4 g
IAA	0.1 mg
Zeatin	2.5 mg
pH	5.8

Medium H	1 liter
MS salts and organics	4,4 g
Sucrose	10 g
Vit. NN stock	1 ml
NAA	0.01 mg
Zeatin	2.0 mg
GA ₃	0.1 mg
pH	5.8
Gelrite	2.5 g

Plasmolysis solution	1 liter
D-sorbitol	91.1 g / 0.5 M

Wash solution*	1 liter
Macro stock	10 ml

CaCl ₂ stock (2 M)	3 ml
Iron stock	10 ml
Micro stock	1 ml
NaCl	14.03 g
NAA	2 mg
BAP	0.5 mg
pH	5.6

Sucrose solution	1 liter
Sucrose	119.8 g

Transformation buffer 1	500 ml
Mannitol	17.3 g
CaCl ₂ * 2H ₂ O	7.34 g
MES	2.5 g
pH	5.6

Transformation buffer 2	500 ml
Mannitol	45.5 g
MgCl ₂ * 6H ₂ O	1.52 g
MES	500 mg
pH	5.6

PEG solution	100 ml
PEG 4000	25g
Mannitol stock (0.8 M)	50 ml
Ca(NO ₃) ₂ stock (2 M)	5 ml

Alginate solution	1 liter
Alginic acid-Na salt	28 g
D-sorbitol	72.88 g

Setting agar	1 liter
Sorbitol	72.88 g
CaCl ₂ * 2H ₂ O	7,351 g
Phyto agar	8 g

Floating solution	1 liter
Sorbitol	72.88 g
CaCl ₂ * 2H ₂ O	7,351 g

Releasing solution	1 liter
Na-citrate	5.88 g
Sorbitol	91.1 g

Stocks solutions

Fluorescein stock	1 ml
Fluoresceine diacetate	5 mg
Acetone	1 ml

Macro stock	1 liter
KNO ₃	74 g
MgSO ₄ , 7H ₂ O	49,2 g
KH ₂ PO ₄	3,4 g
Store at 4°C	

Iron stock	100 ml
Na ₂ EDTA	140 mg
FeSO ₄ , 7H ₂ O	190 mg
Store at 4°C	

Micro stock	100 ml
H ₃ BO ₃	150 mg
MnSO ₄ * H ₂ O	500 mg
ZnSO ₄ * 7H ₂ O	100 mg
Na ₂ MoO ₄ * 2H ₂ O	12 mg
CuSO ₄ * 5H ₂ O	1,2 mg
CoCl ₂ * 6H ₂ O	1.2 mg
KI	38 mg
Store at 4°C	

Vitamins NN stock	50 ml
Glycine	100 mg
Myo-Inositol	5000 mg
Thiamine-HCl	25 mg
Pyridoxine-HCl	25 mg
Nicotinic acid	250 mg
Folic acid	25 mg
Biotin	2.5 mg
Store at -20°C	

Vit. mix 1 stock	100 ml
Pantothenic acid	50 mg
Choline chloride	50 mg
Ascorbic acid	100 mg
p-Aminobenzoic acid	1ml
Nicotinic acid	50 mg
Pyridoxine-HCl (Vitamin B6)	50 mg
Thiamine-HCl	500 mg
Store at -20°C	

Vit. mix 2 stock	100 ml
Folic acid	20 mg
Biotin – H1 vit	0.5 mg
Cyanocobalamin – B12 vitamin	1 mg
Store at -20°C	

Vit. mix 3 stock	100 ml
Cholecalciferol – D3 vitamin	0.5 mg
Store at -20°C	

Sugars stock	100 ml
Sorbitol	625 mg
Sucrose	625 mg
D(-)Fructose	625 mg
D(-)Ribose	625 mg
D(+)Xylose	625 mg
D(+)Mannose	625 mg
L(+)Rhamnose monohydrate	625 mg
D(+)Cellobiose	625 mg
Myo-Inositol	250 mg
Store at 4°C	

Organic acids stock	100 ml
Pyruvic acid	100 mg
Fumaric acid	200 mg
Citric acid monohydrate	200 mg
L-Malic acid	200 mg
Store at 4°C	

GOI cDNA sequence (1854 bp)

(from 1-856 bp)

ATGGAGCAAGAGATGGCGGAGTATCTTCCTTTAGATCGAGGATCTAAACGGAGGATTACAGAGAAGCAAACCTCTTCACATTCTTCTCCGATTCCAATTAAGTAG
TACCTCGTTCTCTACCGCCTCATAGAAGGAAATCTAGCTCCTAGATTTTGCTCCTAAGTCTCTTCGTTTGAAGAAGTGAAGAAGAGGCTAAGGTTAATTTTCATC

gRNA_1

Exon1

10 20 30 40 50 60 70 80 90 100

AAAAAAGGGTGGATTTATTACCATGCCTTTTATCATAGCAAATGAGGCACTGGAGAGTGTGGCGAGCTATGGACTTTTACCAAATATGACAAATTATCTGATGGGAC
TTTTTCCCACCTAAATAATGGTACGGAAAATAGTATCGTTTACTCCGTGACCTCTCACACCGCTCGATACCTGAAAATGGTTTATACTGTTTAATAGACTACCTG

Exon1

Exon2

110 120 130 140 150 160 170 180 190 200 210

AATACAGGATGGGGTTTACTACTGCTCAAAATCTTCTGTTTTCTGGTCAGCTACTACCAATTTTTGCCTATTGTTGGAGCTGTTGTTGCTGATTCAATTTAGGT
TTATGTCTACCCCAATGATGACGAGTTTTAGAAGACAAAAGACCAGTCGATGATGGTTAAAAACGGATAACAACCTCGACAACAACGACTAAGTATAAATCCA

gRNA_3

Exon2

220 230 240 250 260 270 280 290 300 310 320

CGATTCTTACTATTGGCCTTGGTTCCATCTTCAGTTTCTGGGATCAACAGTGTGTGGTTAACAGCAATGATTCCGAAAGCCAGGCCTCCGCCTTGCAATCAAAC
GCTAAGGAATGATAACCGGAACCAAGGTAGAAGTCAAAGGACCCTAGTTGTCAACAACCAATTGTCGTTACTAAGGCTTTCGGTCCGGAGGCGGAACGTTAGTTTG

Exon2

Exon3

330 340 350 360 370 380 390 400 410 420

AGGACAGGCTTGTAATTCTACAACGGGACCACAATACATGCTCTTGTTTTCTCGTTTCTGCTCTTGTCGAATTGGTGCTGGAGGTATAAGACCATGTTCTTTAGCCT
TCCTGTCCGAACATTAAGATGTTGCCCTGGTGTATGTACGAGAACCAAAAGAGCAAAGACGAGAACAGTTAACCACGACCTCCATATTCTGGTACAAGAAATCGGA

gRNA_4

Exon3

430 440 450 460 470 480 490 500 510 520 530

TTGGTGCAAACCAAGTTTGACAAGGGAGGTAGCGATCCCAACAAACAGACAGTGTGGAGAGCTTCTTTGCCTGGTATTATACTTCATCTGTAGTCTCTGTTCTGATT
AACCACGTTTGGTCAAACCTGTTCCCTCCATCGCTAGGGTTGTTGTCTGTCAACCTCTCGAAGAAACGGACCATAATATGAAGTAGACATCAGAGACAAGACTAA

Exon3

540 550 560 570 580 590 600 610 620 630 640

GCCCTAACGGGTATCGTTTACCTTCAAGACAGACTTGGGTGAAAAATAGGTTTTGGAGTTCCTGCAATTCTCATGTTCTTATCCGCGTTGTTTTCTTCTTGCTTC
CGGGATTGCCCATAGCAAATGGAAGTTCTGTCTGAACCCACCTTTTATCCAAACCTCAAGGACGTTAAGAGTACAAGAATAGGCGCAACAAAAAGAAGGAACGAAG

Exon3

650 660 670 680 690 700 710 720 730 740

TCCGTTTTATATCAAGCCAAAGGTTTCGCTCAAATGTGTTTGCCAGCTTTATACGAGTAATTGTGGTTGCCTTCAAGAATAGGAACTACATTACCCCAATCAGAACT
AGGCAAAATATAGTTTCGGTTTTCCAAGCGAGTTTACACAAACGGTCGAAATATGCTCATTAAACACCAACGGAAGTTCTTATCCTTTGATGTAATGGGGTTAGTCTTGA

Exon3

750 760 770 780 790 800 810 820 830 840 850

GOI cDNA sequence (1854 bp) (from 857-1712 bp)

CTGATTATCATCACAAGAATGGTTCAGGACCTCAAGTGCCAACGGAGAAATTGAGATTCTTAAACAAAGCTTGCATCATTTAAAGCCCTGAAGATGTTAATCCAAAC
GACTAATAGTAGTGTCTTACCAAGTCTGGAGTTCACGGTTGCCTCTTAACTCTAAGAATTTGTTTCGAACGTAGTAATTTTCGGGACTTCTACAATTAGGTTTG

gRNA_5

Exon3

860 870 880 890 900 910 920 930 940 950 960

GGAGTTGCAGCCAATCCATGGAACCTTTGCACAGTGGAGCAAGTTGAGGAGCTAAAAGCCCTCGTTAGAATCGTGCCATTGTGGTCAACAGGGATCATGATATCAAT
CCTCAACGTCGGTTAGGTACCTTGAAACGTGTACCTCGTTCAACTCCTCGATTTTCGGGAGCAATCTTAGCACGGTAACACCAAGTTGTCCTTAGTACTATAGTTA

Exon3

970 980 990 1,000 1,010 1,020 1,030 1,040 1,050 1,060 1,070

AAACTTGAGCCAAAGTTCATTCCCACTACTACAAGTCTAATCCATGAATAGACATCTAACTAAAGGATTCCAAATTCAGCAGGGTCATTGCGGATGTTTTTGATGA
TTTGAACCTCGTTTTCAAGTAAGGGTGATGATGTTTCGAGTTAGGTACTTATCTGTAGATTTCCTAAGGTTAAGGTCGTCCAGTAAGCCCTACAAAACTACT

Exon3

1,080 1,090 1,100 1,110 1,120 1,130 1,140 1,150 1,160 1,170

TTGCATTAACAATTTGGGTATTACTGTATGACCGCGTGATGCTTCCATTGGCATCAAAGATCAAAGGAAGACCAGTTCGTCTAAAACCTATAGTCAGAATGGGACTT
AACGTAATTGTTAAACCCATAATGACATACTGGCGCACTACGAAGTAACCGTAGTTTCTAGTTTCCTTCTGGTCAAGCAGATTTTGGATATCAGTCTTACCTGAA

Exon3

1,180 1,190 1,200 1,210 1,220 1,230 1,240 1,250 1,260 1,270 1,280

GGTATATTCGTGTCTTGCATGTCCATGGTAGTCTCTGGTATTATCGAAAATATTTCGACGAAGAAGAGCAATCAGTGAAGGGCTGTTGAACAACTCGCAGGGGTTGGT
CCATATAAGCACAGAACGTACAGGTACCATCAGAGACCATAATAGCTTTTATAAGCTGCTTCTTCTCGTTAGTCACTTCCCACAACCTGTTGAGCGTCCCCAACCA

Exon3

1,290 1,300 1,310 1,320 1,330 1,340 1,350 1,360 1,370 1,380 1,390

GGAGATGTCAGCAATGTGGCTCATTATACCAACAGTTTAAACGGTATAGCAGAGGCGTTGAACGCGATTGGCGCCACAGAGTTCTATTATTTCAGAGCTCCCAAAGA
CCTCTACAGTCGTTACACCGAGTAATATGGTTTTGTCAAATTTGCCATATCGTCTCCGCAACTTGCGCTAACC GCGGTGTCTCAAGATAATAAGTCTCGAGGGTTTCT

Exon3

1,400 1,410 1,420 1,430 1,440 1,450 1,460 1,470 1,480 1,490

GTATGTCAAGTATTGCATCAGCTCTTTTAGGACTGGGAATGGCAGTTGCAAATCTTTTAGCAAGTGTTGTTTTGAGTGCTGTGGATAAGTACACGAAAGGAGAAGGG
CATACAGTTCATAACGTAGTCGAGAAAATCCTGACCCTTACCGTCAACGTTTAGAAAATCGTTCACAACAAAACCTACGACACCTATTCATGTGCTTTCCTCTCCC

Exon3

1,500 1,510 1,520 1,530 1,540 1,550 1,560 1,570 1,580 1,590 1,600

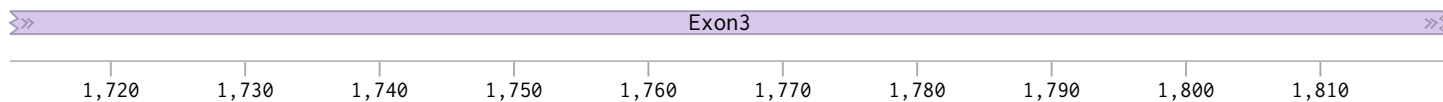
AAAGAAAGTTGGATTTCAAGCAATATCAACAGGGGACACTATGAGTATTACTACTGGCTTCTTGCTCTAATGACAGGTTTTAATCTGCTTTATTTTGTGGTTTGTG
TTTCTTTCAACCTAAAGTTCGTTATAGTTGTCCCTGTGATACTCATAATGATGACCGAAGAACGAGATTACTGTCCAAAATTAGACGAAATAAAACACCAACAAC

Exon3

1,610 1,620 1,630 1,640 1,650 1,660 1,670 1,680 1,690 1,700 1,710

GOI cDNA sequence (1854 bp) (from 1713-1854 bp)

CTGGCAATATGGACCTTCTGTTGATGTTGACATCACTATGAGAATGATGGAACCTAGCGACGATGAAGATGACGATGACGATGAAGATAAAGATGATTTGCCTAAGA
GACCGTTATACCTGGAAGACAACCTACAACCTGTAGTGATACTCTTACTACCTTGGATCGCTGCTACTTCTACTGCTACTGCTACTTCTATTTCTACTAAACGGATTCT



AGAATAAATCTACACCTGACTTGAATTCCTGCTAG
TCTTATTTAGATGTGGACTGAACTTAAGGACGATC

