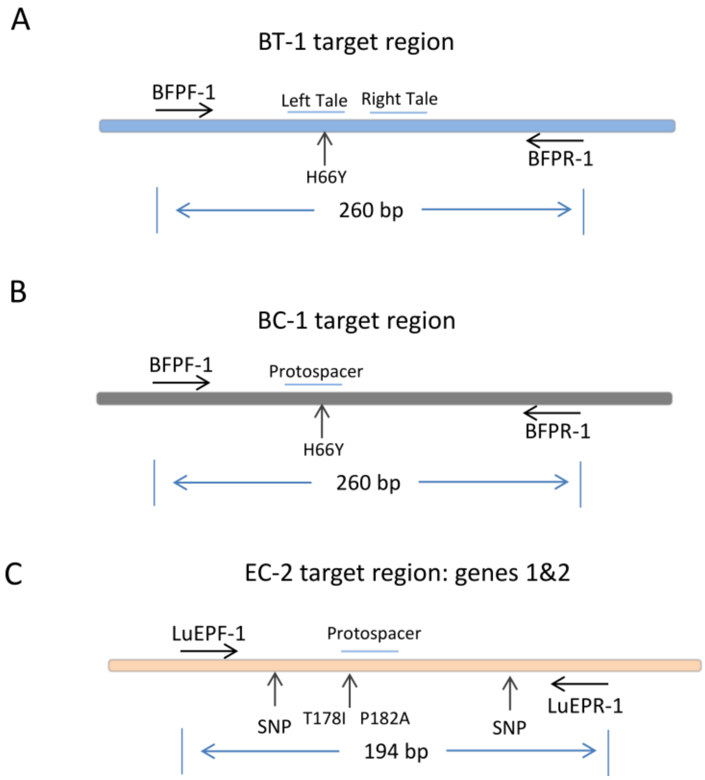
SUPPLEMENTAL INFORMATION



Supplemental Figure S1. Diagram showing the methodology for amplicon deep sequencing analysis. A, Primers BFPF-1 and BFPR-1 amplify a region 260 bp in length that flanks the BT-1 TALEN binding region. B, Primers BFPF-1 and BFPR-1 amplify a region 260 bp in length that flanks the BC-1 target region. C, Primers LuEPF-1 and LuEPR-1 amplify a region 194 bp in length from a conserved region in genes 1 and 2. SNPs within the amplicon provide gene 1 and 2 specificity.

Supplemental Table S1. *Sequence of ssODNs used in this study*

ID	Sequence (5' to 3')	Chemistry
BFP/41	G*C*T*GAAGCACTGCACGCCGTAGGTGAAGGTGGTCACGA*G*G*G	(*) = 3PS
BFP/41/NT	G*C*T*GAAGCACTGCACGCCGTGGGTGAAGGTGGTCACGA*G*G*G	(*) = 3PS
BFP/101	G*T*C*GTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTGAAGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGG*T*G*G	(*) = 3PS
BFP/201	A*A*G*ATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTGAAGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCG*C*C*C	(*) = 3PS
EPSPS/144*	VCGGTCGGTAAACTGGCGAAGAACGATATTGAACTTTTCCTTGGAAATGCTGGAATAGCTATGCGTGCGCTGACAGCTGCTGTAACAGCCGCTGGAGGCAACTCAAGGTCCCTTCCCTCAACTCCTTCCAGCCTTTCAGCTTCTTH and VCGGTCGGTAAACTGGCGAAGAACGATATTGAACTTTTCCTTGGAAATGCTGGAATAGCTATGCGTGCGCTGACAGCTGCTGTAACAGCCGCTGGAGGCAACTCAAGGTTCCTTCCCTCAACTCCTTCCAGCCTTTCAGCTTCTTH	V=CY3; H=3'DMT dC CPG

*EPSPS/144 consists of an equimolar mixture of two ssODNs, each containing sequence specific SNPs for *EPSPS* gene 1 and 2. 3PS- 3 consecutive phosphorothioate linkages. Cy3- cyanine dye 3.

Supplemental Table S2. *TALE binding domain sequence*

TALEN ID	Sequence (5' to 3')
BT-1	Left arm: TGGTCGGGGTAGCGGCTGA Right arm: TCGTGACCACCTTCACCCA

Supplemental Table S3. *Primers used in this study*

ID	Sequence 5'-3'	Species
BFPF-1	TAAACGGCCACAAGTTCAGC	*Arabidopsis*
BFPR-1	GGACGACGGCAACTACAAGACC	*Arabidopsis*
LuEPF-1	GCATAGCAGTGAGCAGAAGC	Flax
LuEPR-1	AGAAGCTGAAAGGCTGGAAG	Flax
Off-1FA	GGAAGCAAACAGGTGACAGC	*Arabidopsis*
Off-1RA	CGTATTTAGCCTCATCCAATGC	*Arabidopsis*
Off-2FA	AAGGCTCCTCCAACTTCACC	*Arabidopsis*
Off-2RA	TTCTCTGACTCTGATGGAGACC	*Arabidopsis*
Off-3FA	CCCTTGGTGCAACATAAACC	*Arabidopsis*
Off-3RA	GCGATGAATTTGAATTTTGACC	*Arabidopsis*
Off-4FA	TTCGGGTTTAACGGGACAG	*Arabidopsis*
Off-4RA	CGATTCCGGTAATTCACATTG	*Arabidopsis*
Off-5FA	AAACCCTAGTGGCAGTTTCG	*Arabidopsis*
Off-5RA	CGGTGGAAGCCCTGTTTAT	*Arabidopsis*
Off-1FF	CAAGGCTAATTAGACTTAGATGATGTGG	Flax
Off-1RF	GGTGCACCGCC	Flax
Off-2FF	CAAGGCTAATTAGACTTAGATGATGTGG	Flax
Off-2RF	GGTGCACCGCC	Flax
Off-3FF	GCCATCATCGCCCTTTAAGC	Flax
Off-3RF	TGGTGTTTTGCTCTGTGAACG	Flax
Off-4FF	GCCATCATCGCCCTTTAAGC	Flax
Off-4RF	TGGTGTTTTGCTCTGTGAACG	Flax
Off-5FF	GCCATCATCGCCCTTTAAGC	Flax
Off-5RF	TGGTGTTTTGCTCTGTGAACG	Flax
Off-6FF	GCCATCATCGCCCTTTAAGC	Flax
Off-6RF	TGGTGTTTTGCTCTGTGAACG	Flax
Off-7FF	GAAAGAAGGCACTCTCAGAACATAC	Flax
Off-7RF	TGAATTTTGCTATCCTCTTCCCAATTTG	Flax
Off-8FF	CGTACGTTGTCAAGAAGTGACC	Flax
Off-8RF	ACCAAGACGGTAGTGGATGTC	Flax

Supplemental Table S4. *Protospacer sequence of sgRNAs used in this study*

CRISPR/Cas9 ID	Sequence 5'-3'
BC-1	GTCGTGACCACCTTCACCCA
EC-2	GCTGTTACAGCAGCTGTCAG

G in red font altered in the sgRNA sequence to accommodate Pol III promoter

Supplemental Table S5. *BC-1 off-targets*

Off-target ID	*Arabidopsis* Chrom #	Position	Off target sequence	# of mismatches	Gene
Off-1	chr1	13067189	gTtGTtgCCACCTTCAaCCAAGG	5	intergenic
Off-2	chr2	19145890	CaCGTccCCACCaTCtCCCAAGG	5	uncharacterized protein
Off-3	chr5	17191806	tTCaTcACCAgCTTCACCaATGG	5	uncharacterized protein
Off-4	chr4	4803635	tTCGTGctCACCTTCACggATGG	5	Cellulose-synthase like
Off-5	chr4	14554739	CTCGaacCCACCTTCAgCaAAGG	5	polyamine oxidase 5
On	NA	NA	CTCGTGACCACCTTCACCCACGG	NA	BFP transgene

Supplemental Table S6. *Off-target analysis of flax plant A23*

Off-Target ID	Scaffold or Locus ID[a]	Position[b]	Off Target Sequence[c]	# of Mismatches	Mutations detected[d]
Off-1	C7813595	197-219	ccgGTTACAGCAGCaGTCgGCGG	5	-
Off-2	Lus10030959.g	243476-243460	ccgGTTACAGCAGCaGTCgGCGG	5	-
Off-3	Scaffold 155	681644-681624	tcaaaagCtGCAGCTaTCAGTGG	9	-
Off-4	Lus10036882.g	1067934-1067911	tcaaaatCtGCAGCTGTCAGTGG	8	-
Off-5	Scaffold 107	1077588-1077568	tcaaaatCtGCgGCTGTCAGTGG	9	-
Off-6	Scaffold 743	195079-195059	tcaaaatCtGCgGCTGTCAGTGG	9	-
Off-7	Scaffold 208	238604-238626	aaggacACAGCAGCTGTCgGTGG	7	-
Off-8	Scaffold 2252	38795-38773	accaaacgAGCAGCTGTCAGAGG	8	-
On	Lus10000788.g	19227-19249	GCTGTTACAGCAGCTGTCAGCGG	0	+

[a] Scaffold or locus ID from Phytozyme 10.2

[b] Protospacer position within scaffold

[c] Red lowercase bases are mismatches to the EC-2 protospacer

[d] Mutations determined by sequencing; On-target mutations are T178I and P182A

Supplementary Sequences

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Flax EPSPS gene 1
Locus name Lus10030282
ATGGCGCTAGTTACCAAAATCTGCGGCGGAGCTAATGCTGTTGCTCTCCCCGCTACATTTGGAACTCGAA
GAACAAAATCCATATCCTCGTCGGTCTCGTTTAGATCTTCAACATCTCCTCCGAGTTTGAAGCAACGCCG
ACGATCGGGCAATGTAGCCGCCGCCGCCGCCGCTCCTCTTCGTGTTTCGGCATCGCTCACCACGGCAGCT
GAGAAGGCTTCCACGGTGCCGGAGGAGGTCGTGCTGCAGCCTATTAAAGACATATCGGGGATTGTGACTC
TGCCTGGTTCTAAATCTCTCTCGAATCGGATTCTCCTTCTCGCCGCACTCTCTGAGGGTACAACTGTGGT
GGACAACTTGTTGAACAGTGATGATGTTCACTACATGCTTGGAGCATTGAAAACACTCGGACTAAATGTG
GAGCATAGCAGTGAGCAGAAGCGCGCCATTGTTGAAGGTTGTGGTGGTGTGTTTCCGGTCGGTAAACTGG
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CGAAGAACGATATTTTTCCTCATGCTTTCGTCTACATACTCGATGGGGTACCACGAATGAGAGAGAGACC
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CCCGTCCATGTAAATGGACAGGGAGGCTTGCCAGGTGGAAAGGTTAAGCTTTCCGGATCAATTAGTAGTC
AATACTTGACTGCTTTGCTGATGGCTGCTCCTTTGGCCCTCGGAGATGTGGAAATCGAGATTGTTGATAA
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TTGTGCCGATGTTCCGGTCACCATAAGGGACCCTGGCTGCACTAAGAAGACATTTCCAGATTACTTTGAA
GTCCTGGAAAGGTACACCAAGCATTGA

Flax EPSPS gene 2
Locus name Lus10000788
ATGGCGCAAGTTACCAAAATCTGCGGCGGAGCTAATGCAGTTGCTCTCCCCGCTACATTTGGAACTCGAA
GAACCAAATCCATATCCTCCTCGGTCTCATTTAGATCTTCAACATCTCCTCCGAGTTTGAAGCAACGTCG
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GCTGAGAAGGCTTCCACGGTGCCGGAGGAGATCGTGCTGCAGCCTATTAAAGACATATCGGGGATTGTGA
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