2012

High-efficiency TALEN-based gene editing produces disease-resistant rice

Ting Li
Iowa State University, tingli99@iastate.edu

Martin H. Spalding
Iowa State University, mspaldin@iastate.edu

Donald P. Weeks
University of Nebraska–Lincoln,, dweeks1@unl.edu

Bing Yang
Iowa State University, byang@iastate.edu

Published in *Nature Biotechnology* 30 (2012), pp. 390–392; doi: 10.1038/nbt.2199 Published online May 7, 2012.

Supplementary information is presented following the References.

Correspondence

High-efficiency TALEN-based gene editing produces disease-resistant rice

Ting Li,[1] Bo Liu,[1] Martin H. Spalding,[1] Donald P. Weeks,[2] and Bing Yang[1]

1. Department of Genetics, Development & Cell Biology, Iowa State University, Ames, Iowa, USA
2. Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, Nebraska, USA
Corresponding author — Bing Yang, email byang@iastate.edu

To the Editor:
Transcription activator–like (TAL) effectors of *Xanthomonas oryzae* pv. *oryzae* (Xoo) contribute to pathogen virulence by transcriptionally activating specific rice disease-susceptibility (*S*) genes[1, 2]. TAL effector nucleases (TALENs)—fusion proteins derived from the DNA recognition repeats of native or customized TAL effectors and the DNA cleavage domains of FokI[3, 4, 5]—have been used to create site-specific gene modifications in plant cells[6, 7], yeast[8], animals[9, 10, 11, 12] and even human pluripotent cells[13]. Here, we exploit TALEN technology to edit a specific *S* gene in rice to thwart the virulence strategy of *X. oryzae* and thereby engineer heritable genome modifications for resistance to bacterial blight, a devastating disease in a crop that feeds half of the world's population.

We targeted the rice bacterial blight susceptibility gene *Os11N3* (also called *OsSWEET14*) for TALEN-based disruption. This rice gene encodes a member of the SWEET sucrose-efflux transporter family and is hijacked by *X. oryzae* pv. *oryzae*, using its endogenous TAL effectors AvrXa7 or PthXo3, to activate the gene and thus divert sugars from the plant cell so as to satisfy the pathogen's nutritional needs and enhance its persistence[2, 14]. The *Os11N3* promoter contains an effector-binding element (EBE) for AvrXa7, overlapping with another EBE for PthXo3 and with the TATA box (Figure 1a and Supplementary Figure 1). We deployed two pairs of designer TALENs (pair 1 and pair 2) independently to induce mutations in these overlapping EBEs of the *Os11N3* promoter and thus to interfere with the virulence function of AvrXa7 and PthXo3, but not the developmental function of *Os11N3* (Supplementary Figure 1 and Supplementary Note). The TALE repetitive regions used for nuclease fusions included the native AvrXa7 and three designer TALE repetitive regions custom synthesized using a modular

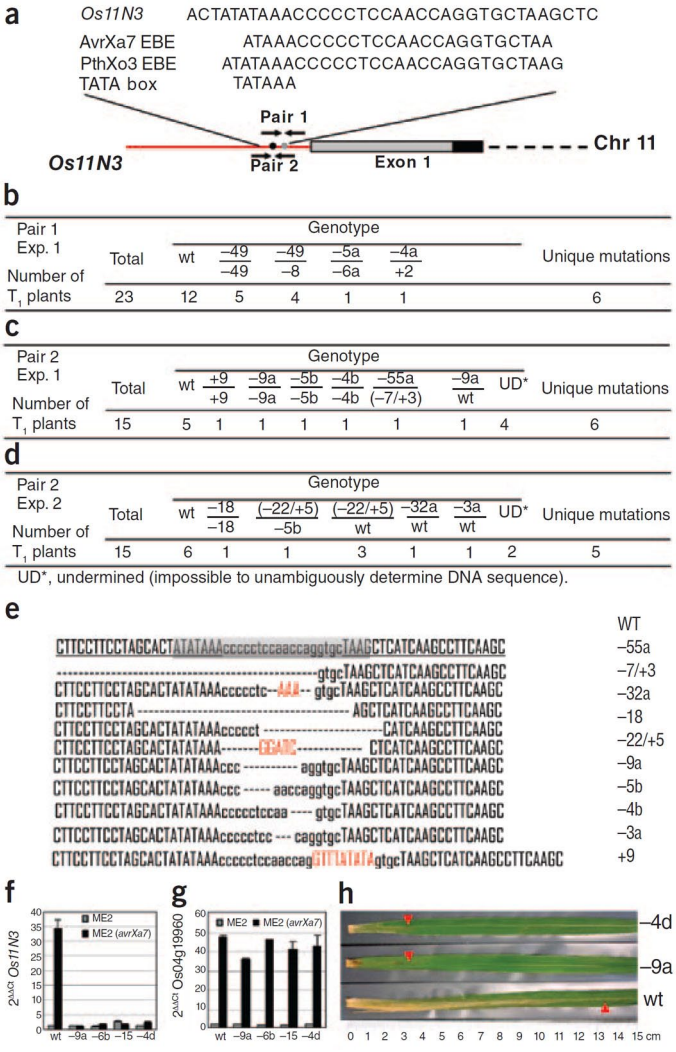


Figure 1. High-efficiency targeted gene editing using TALENs. (**a**) Overlapping elements targeted by two pairs (1 and 2) of designer TALENs in the *Os11N3* promoter. (**b–d**) Genotypes of progeny (T_1) of primary transgenic plants (T_0) derived from TALEN-expressing embryonic cells from three independent transformation experiments (Exp.). Each of the two alleles of an individual plant are designated as being wild type (wt) or as having a nucleotide insertion (+) or a deletion (−) and are separated top and bottom by a dividing line. The designation "−55/(−7/+3)" indicates that one allele contains a deletion of 55 bp and that the other allele has both a deletion of 7 bp and an insertion of 3 bp. (**e**) Sequences of *Os11N3* mutations induced by the pair 2 TALENs with deletions (dashes) and insertions (red letters). TALEN-binding sequences are underlined in wt and the overlapping EBEs are shaded in gray. (**f**,**g**) Expression of *Os11N3* and Os04g19960 induced by AvrXa7 in plants of different genotypes. Quantitative reverse transcription (RT)-PCR was performed with RNA derived from treatments of nonpathogenic Xoo strain ME2 and pathogenic ME2(*avrXa7*). $2^{-\Delta\Delta Ct}$ is a measure of transcript abundance for a selected gene (*Os11N3* in **f** or Os04g19960 in **g**) relative to the abundance of transcripts produced from a constitutively expressed gene (*OsTFIIAg5*), as determined by relative PCR cycle thresholds (*Ct*). (**h**) Resistance phenotype displayed by two T_2 mutant plants compared with the disease susceptibility phenotype of a nontransgenic wt rice plant.

assembly method[8]. Each designer TALEN contained 24 repeat units for recognition of a specific set of 24 contiguous nucleotides at the target sites (Supplementary Figure 1).

For each pair of TALEN genes, one TALEN gene (half of the pair) was under the control of the 35S promoter of cauliflower mosaic virus and the other gene was driven by the maize ubiquitin 1 promoter, comprising a specific TALEN pair in a single plasmid (Supplementary Figure 2). Each plasmid also contained a marker gene for hygromycin resistance. These constructs were introduced into rice embryonic cells using *Agrobacterium tumefaciens*, and individual transformant cells were selected, propagated and regenerated into whole plants (T_0). The *Os11N3* promoter regions from a number of independent hygromycin-resistant callus lines and the segregating progeny (T_1) of self-pollinated T_0 plants were amplified using the polymerase chain reaction (PCR) and sequenced to detect potential sequence alterations. For TALEN pair 1 genes, two of five examined callus lines contained biallelic mutations (Supplementary Figure 3). Of 23 randomly selected T_1 progeny produced from self-pollination of 7 independent T_0 plants transformed with TALEN pair 1 genes, about half (48%) carried mono- or biallelic mutations (including the four mutations detected in the two previously examined callus lines; Figure 1b). Approximately two-thirds (63%) of the randomly selected T_1 plants (n = 30) generated from self-pollination of 66 independent T_0 plants from the two independent transformation experiments carried mutations that were induced by the TALEN pair 2 genes (Figure 1c,d). In total, 16 distinct mutations, including 6 that were homozygous, were detected in 53 T_1 plants from TALEN pair 1 and pair 2. The majority of these mutations were small deletions that left the TATA box intact, with the exception of two deletions in heterozygous lines that also contained a wild-type allele (Figure 1e and Supplementary Figure 4). Bacterial infection assays using the leaf-tip clipping method on other T_1 plants (n = 627) generated from TALEN pair 2 (experiment 1) and not previously genotyped demonstrated that approximately 48% of the treated plants showed resistance to infection by pathogenic Xoo as evidenced by the length of leaf lesions (1–4 cm for resistance versus 10–14 cm for susceptibility; Supplementary Figure 5). DNA sequence analyses of 27 such Xoo-resistant T_1 plants confirmed the presence of homozygous monoallelic or heterozygous biallelic EBE mutations and revealed 17 additional, distinct mutant haplotypes (Supplementary Figure 6). All mutant plants were morphologically normal compared to wild-type plants, indicating that the developmental function of *Os11N3* was not disrupted.

Forty plants from the second generation (T_2) of three self-pollinated T_1 plants were also genotyped by sequencing to determine the heritability of three TALEN-generated mutations, all of which, whether homozygous or heterozygous, were passed on to T_2 plants (Supplementary Figure 7).

To determine the effects of TALEN-directed mutations, we investigated whether the pathogenic strain of Xoo that is dependent on AvrXa7 or PthXo3 for virulence is able to either induce the modified *Os11N3* gene in homozygous T_2 plants or cause disease. The modified *Os11N3* gene was no longer inducible by AvrXa7 or PthXo3 delivered by the pathogenic strain of the bacterium (ME2(*avrXa7*) or ME2(*pthXo3*)) in T_2 plants homozygous for either the 9-, 6-, 15- or 4-bp deletion (Figure 1f for AvrXa7, Supplementary Figure 8a for PthXo3). The loss of induction was specific to *Os11N3*, as the induction of Os04g19960, a transposon coding gene collaterally targeted by AvrXa7, was not prevented (Figure 1g). Similarly, the induction of another S gene (*Os8N3*, also known as *OsSWEET11*) by PthXo1 in the T_2 mutant plants remained unaffected (Supplementary Figure 8b). These TALEN-modified T_2 plants also showed strong resistance to infection of the AvrXa7- or PthXo3-dependent Xoo strains but not the PthXo1-dependent pathogenic Xoo strain as determined from symptoms (Figure 1h for AvrXa7) and by quantitative measurement of the lengths of leaf lesions in a standard pathogenesis assay described in Supplementary Methods (Supplementary Figure 9).

We also investigated the possibility of using genetic segregation to obtain genetically modified rice lacking any selection marker and TALEN gene. The PCR assay using primers for amplification of the hygromycin resistance gene and for amplification of the TALEN genes failed to detect the presence of either gene in 5 out of 37 T_1 plants that contained the desired genetic modifications in the *Os11N3* promoter and that were disease resistant (Supplementary Figure 10). Although

these data clearly demonstrate the absence of intact TALEN and hygromycin-resistance genes, further sequencing of the genomes of several mutants and the Kitake parental line will be needed to conclusively demonstrate that all of the transgene fragments have been removed.

The rice *Os11N3* gene is induced by 32 of 40 Xoo strains collected worldwide (T. Li and B. Yang, unpublished data). However, polymorphisms in the *Os11N3* gene that prevent induction by AvrXa7- and/or PthXo3-dependent Xoo strains and also confer disease resistance have not been identified in rice germplasm. The approaches described here for precisely and efficiently editing the disease susceptibility elements in *Os11N3* and for the subsequent removal of transfer DNA (T-DNA) sequences by classic genetics likely can be applied directly to elite rice varieties to simultaneously or sequentially edit multiple susceptibility genes (for example, *Os11N3* and *Os8N3*), leading to resistance to the major forms of bacterial blight. Present methods using TALEN-based technology in rice should be easily modified for application to other plant species and, thus, hold substantial promise in facilitating gene modification–based research and crop improvement.

Acknowledgments — We thank D. Wright and C. Yao for helpful suggestions, L. Xue and J. Luo for technical assistance, and F.F. White and S. Howell for critical reading of the manuscript. This work was supported by grants from the US National Science Foundation (0820831 to B.Y., MCB-0952323 to M.H.S., and MCB-0952533 and EPSCoR grant 1004094 to D.P.W.).

Author contributions — B.Y. and T.L. conceived the study. T.L. and B.L. performed the experiments. B.Y., T.L., M.H.S. and D.P.W. analyzed the data. All authors contributed to the writing of the paper.

Competing financial interests — T.L., B.L. and B.Y. are inventors on a patent application covering TALEN-mediated rice engineering for disease resistance.

References

1. Yang, B., Sugio, A. & White, F.F. *Proc. Natl. Acad. Sci. USA* 103, 10503–10508 (2006).
2. Antony, G. *et al. Plant Cell* 22, 3864–3876 (2010).
3. Li, T. *et al. Nucleic Acids Res.* 39, 359–372 (2011).
4. Christian, M. *et al. Genetics* 186, 757–761 (2010).
5. Miller, J.C. *et al. Nat. Biotechnol.* 29, 143–148 (2011).
6. Mahfouz, M.M. *et al. Proc. Natl. Acad. Sci. USA* 108, 2623–2628 (2011).
7. Cermak, T. *et al. Nucleic Acids Res.* 39, e82 (2011).
8. Li, T. *et al. Nucleic Acids Res.* 39, 6315–6325 (2011).
9. Sander, J.D. *et al. Nat. Biotechnol.* 29, 697–698 (2011).
10. Huang, P. *et al. Nat. Biotechnol.* 29, 699–700 (2011).
11. Tesson, L. *et al. Nat. Biotechnol.* 29, 695–696 (2011).
12. Wood, A.J. *et al. Science* 333, 307 (2011).
13. Hockemeyer, D. *et al. Nat. Biotechnol.* 29, 731–734 (2011).
14. Chen, L.Q. *et al. Science* 335, 207–211 (2012).

SUPPLEMENTARY INFORMATION

High-efficiency TALEN-based gene editing produces disease-resistance rice

Ting Li[1], Bo Liu[1], Martin H. Spalding[1], Donald P. Weeks[2], Bing Yang[1 *]

[1]Department of Genetics, Development & Cell Biology, Iowa State University, IA 50011, USA;

[2]Department of Biochemistry, University of Nebraska-Lincoln, NE 68588 , USA.

[*] To whom correspondence should be addressed. E-mail: byang@iastate.edu

Table of Contents

Nature Biotechnology: doi:10.1038/nbt.2199

Supplementary Figure 9. Severity of disease damage to wild type and transgenic rice plants caused by AvrXa7-, PthXo3-, and PthXo1-dependent Xoo strains.

Supplementary Figure 10. Removal of T-DNA sequences containing TALEN genes from TALEN-modified rice plants using genetic crossing.

Supplementary Note. Open reading frame sequences of three designer TALENs.

Reference List (1-9)

Nature Biotechnology: doi:10.1038/nbt.2199

Materials and Methods

TALEN design and construction. Two pairs of designer TALENs (Pair 1: dTALEN L1 and dTALEN R1 and Pair 2: TALEN AvrXa7 and dTALEN R2) were used in the present studies. TALEN AvrXa7 was based on a native AvrXa7 TAL effector and contained a truncated transcription activation domain fused at its C-terminus with a wild type FokI DNA cleavage domain [1,2]. The other three TALENs employed in these studies were made using previously described "modular assembly" methods [2]. Such assembly used four different genes encoding TAL effector DNA binding domains that, due to specific codons in position 12 and 13 of the coding sequence, are capable, respectively, of recognizing either an A, G, C or T residue in the DNA sequence of a particular target effector binding element (EBE). Each designer TALEN contained a wild type FokI DNA cleavage domain [1,2]. All TALENs used a complete TAL effector N-terminus. Diagrams showing the structure of each TALEN and the EBE DNA sequence to which it matches are shown in **Supplementary Figure 1**. DNA sequences for the open reading frames of all three designer TALENs are provided in **Supplementary Note**.

Construction of TALEN expression plasmids and rice transformation. The two promoters used to express the paired TALEN genes were the maize ubiquitin 1 promoter (ubi1) and the 35S promoter of cauliflower mosaic virus (35S) [3,4]. One of the paired TALEN genes was cloned downstream of the 35S promoter at BamHI and SpeI sites in a binary vector and the other under the control of the *ubi1* promoter was cloned into the BamHI and SacI sites of an intermediate vector. The ubi1-TALEN gene expression cassette was excised with HindIII and moved into the HindIII site of the binary plasmid containing the 35S-TALEN gene expression cassette. The resultant plasmids were mobilized into *Agrobacterium tumefaciens* strain EHA105 by electroporation. *Agrobacterium*-mediated transformation of the rice cultivar Kitake was conducted according to a previously described protocol [5].

DNA sequencing analysis of regions in the *Os11N3* gene targeted by TALENs. Genomic DNA from individual plants was extracted using the CTAB method as described [6]. Forward primer, 5'- TCCCTTAACTAGGACAACTTGGA-3', and reverse primer, 5'-CCGGATCCAGCCATTGCAGCAAGATCTTG-3', were used to amplify a region of ~ 550 bp

Nature Biotechnology: doi:10.1038/nbt.2199

with the preselected target sites located in the middle. The PCR products from individual plants were sequenced using an internal primer, 5'-CATGGCTGTGATTGATCAGG-3'. Each sequencing chromatogram was manually analyzed for polymorphisms within a trace.

Quantitative RT-PCR analysis of rice gene inducibility. Bacterial inoculums with optical density of 1.0 at 600 nm (OD_{600}) were infiltrated into rice leaves by using needle-less syringe as described [7]. The bacterial strains used were Xoo strain PXO99ME2 (hereafter designated as ME2, a PXO99 derivative strain lacking TAL effector PthXo1 with concomitant loss of strain virulence), ME2(*avrXa7*), ME2(*pthXo3*) and ME2(*pthXo1*), three ME2 transformants containing the respective TAL effector genes *avrXa7*, *pthXo3* and *pthXo1*. Both ME2(*avrXa7*) and ME2(*pthXo3*) induce *Os11N3* and retain virulence, while ME2(*pthXo1*) retains virulence by inducing another S gene (*Os8N3*) [7]. Total RNA of the inoculated portion of leaves was extracted using TRI reagent from Ambion 24 hours after bacterial inoculation. RNA concentration and quality were determined using an ND-1000 Nanodrop spectrophotometer (Nanodrop Technologies). One microgram of RNA from each sample was treated with DNase 1 (Invitrogen) followed by cDNA synthesis using the iScript cDNA synthesis kit (Bio-Rad). cDNA derived from 25 ng of total RNA was used for detection of gene induction by AvrXa7, PthXo3 and PthXo1 using real-time quantitative PCR analyses. PCR was performed on Stratagene's Mx4000 multiplex quantitative PCR system using the iQ SYBR Green Supermix kit (Bio-Rad). In addition to inducing *Os11N3*, AvrXa7 also "collaterally" induces another rice gene Os04g19960, which encodes a putative retrotransposon protein, but is not associated with disease susceptibility in rice. Gene-specific primers for *Os11N3* are 5'-GAGAAGAAGGTAGCTGCATGAGTG-3' and 5'- TCATGGAAGGAACCCTTACAGGTTG-3', primers for Os04g19960 are 5'-AGAAGGCGTAGGCATTCACAT-3' and 5'- ACATTAACACAGCACACGTCAAC-3', and primers for *Os8N3* are 5'-GACTCCATGTCCCCGATCTCC-3' and 5'-CACCACCTCGACCTTGTGCA-3'. The rice general transcription factor *TFIIAγ5* expression was used as an internal control with primers 5'-CTACTCAGCCAATAAATTGATAACTGC-3'and 5'-CAATTTCTACTACTCATCGTTTAG-3'. The average threshold cycle (Ct) was used to determine the fold change of gene expression. The $2^{\Delta\Delta Ct}$ method was used for relative quantification [8].

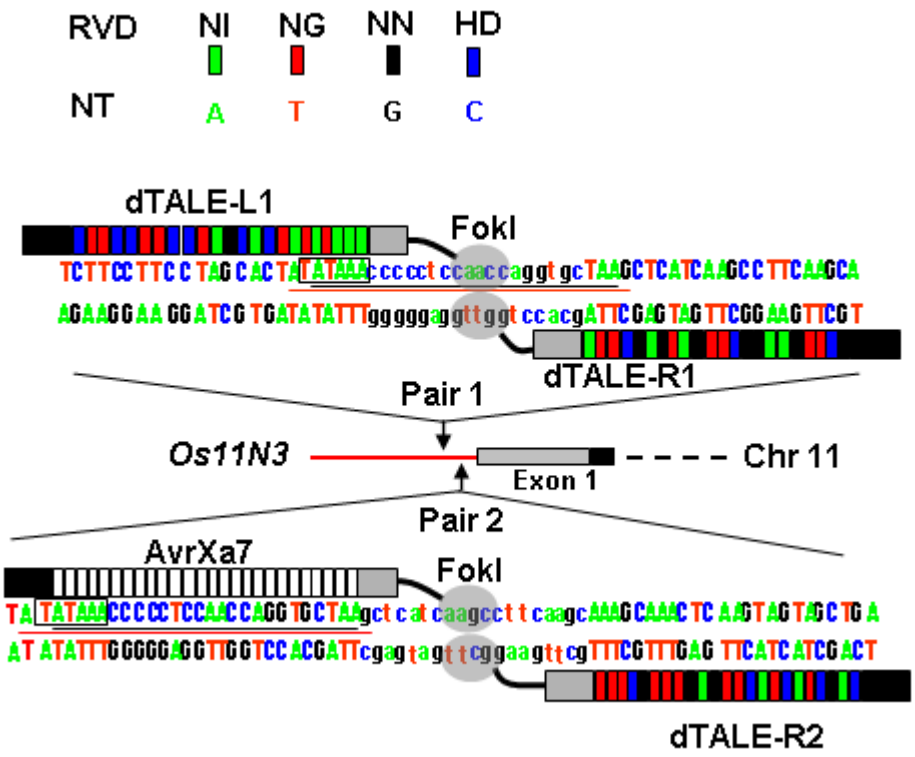
Nature Biotechnology: doi:10.1038/nbt.2199

PCR determination of the presence or absence of T-DNA in genetically modified rice plants. Genomic DNA was extracted as described [6] and used for PCR amplification of fragments from the *Os11N3* gene promoter, hygromycin phosphotransferase (*hpt*) gene and TALEN genes. Primers for the *Os11N3* gene were: forward, 5'-CATGGCTGTGATTGATCAGG-3' and reverse, 5'-CCGGATCCAGCCATTGCAGCAAGATCTTG-3'; primers for the *hpt* gene were: Hyg-F, 5'-CCGCTCGTCTGGCTAAGATC-3' and 35S-R, 5'- CGCTGAAATCACCAGTCTCTC-3'; and primers for the TALEN genes were: FokI-F, 5'-CAGCTAGTGAAATCTGAATTGG-3' and Nos-R, 5'-CATCGCAAGACCGGCAACAGG-3'.

Surveyor nuclease cleavage assay for detection of nucleotide insertions and deletions. Genomic DNA was extracted using the CTAB method as described [6] from individual callus lines and subjected to PCR amplification of the *Os11N3* promoter region (~ 550 bp) using the gene-specific primers (5'- TCCCTTAACTAGGACAACTTGGA-3' and 5'-CCGGATCCAGCCATTGCAGCAAGATCTTG-3'). The Surveyor nuclease (Surveyor mutation detection kit, Transgenomic) was used to treat the PCR products following the manufacturer's instruction. The treated DNA was subjected to electrophoresis in a 1.5% agarose gel and visualized by staining with ethidium bromide.

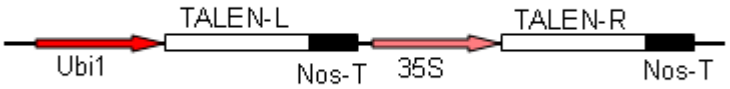
Disease resistance assay. Fully expanded leaves of rice plants were inoculated using leaf tip clipping method. In this previously described procedure [9], scissors blades are immersed in bacterial suspension (OD_{600}=0.5) of *avrXa7*-containing strain PXO86, *pthXo3*-containing strain ME2(*pthXo3*) and *pthXo1*-containing strain PXO99 immediately prior to clipping each target leaf. Symptoms were scored 12-14 days after inoculation by measuring lesion length. Plants were categorized as resistant (R) if lesion lengths were shorter than 4 cm or susceptible if lesions were longer than 8 cm.

Nature Biotechnology: doi:10.1038/nbt.2199



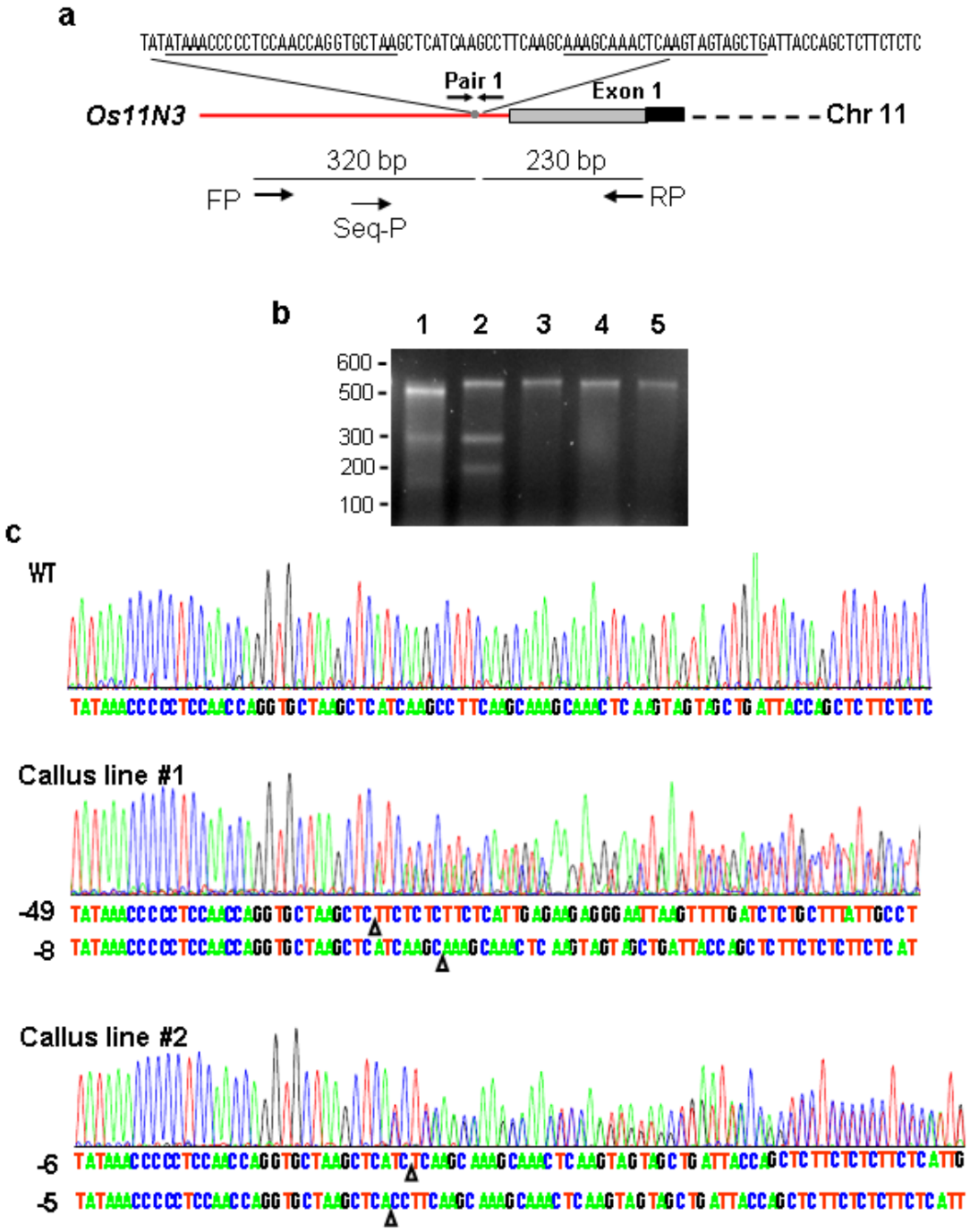
Supplementary Figure 1. TALENs and their DNA targets in the promoter of chromosomal *Os11N3* gene. Four basic modular repeats whose repeat variable di-residue (RVD) (colored blocks) recognizing one nucleotide (NT) in the target site are used to assemble the DNA binding domain of each designer TALEN. The two pairs of nucleases (Pair 1 and 2) are fusions between the DNA cleavage domain of FokI (FokI) and the native (AvrXa7) or customized TAL effector (dTALE). The last 40 amino acids at C-terminus of dTALE-L1 and AvrXa7 are truncated to avoid the inappropriate induction of *Os11N3* by the activation domain. The other two TAL effectors (dTALE-R1 and dTALE-R2) contain the complete C-terminus. All four TAL effectors contain the complete N-terminus. *Os11N3* promoter contains an effector binding element (EBE) for AvrXa7 (underlined in black), an EBE for PthXo3 (underlined in red) and the TATA box (boxed). Lower letters represent regions wherein two FokI domains dimerize and cause a double stranded DNA break.

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 2. Schematic diagram of a two-gene expression cassette in a single binary vector designed for *Agrobacterium*-mediated rice transformation. The expression cassette includes a promoter [maize ubiquitin 1 promoter (Ubi1) (red arrow) to drive expression of the TALEN-L gene (open box), the cauliflower mosaic virus 35S gene promoter (35S) (red arrow) to allow transcription of the TALEN-R gene (open box)] and a gene terminator (Nos-T) (black box).

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 3. Analysis of site-specific mutations within the *Os11N3* gene promoter in T0 callus lines expressing the Pair 1 TALENs. (a) Schematics of the *Os11N3* gene promoter target site with primers (FP & RP) for PCR amplification of promoter sequence (550 bp) and primer (Seq-P) for sequencing the PCR products. (b) Analysis of PCR products derived from five individual callus lines using the Surveyor nuclease cleavage assay (see Methods and Materials) and showing two out of five calli (#1 and #2) with biallelic mutations. This assay is

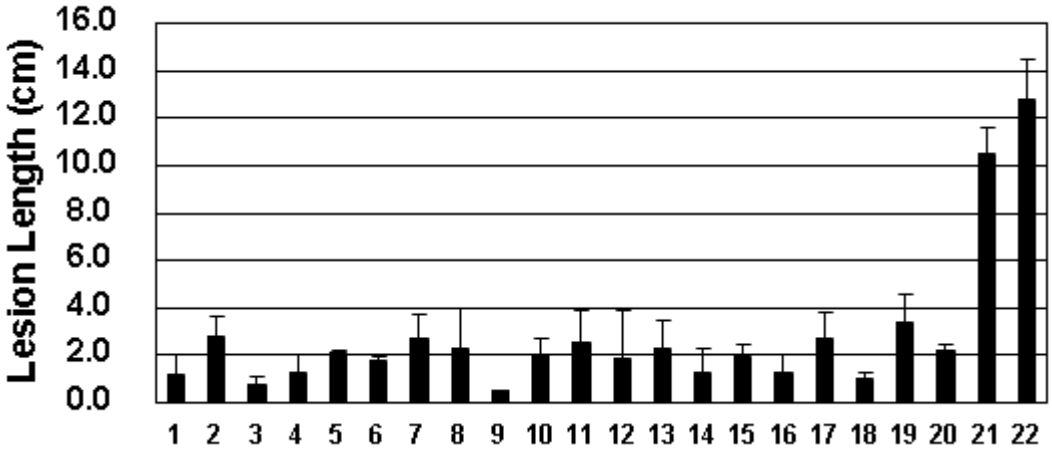
Nature Biotechnology: doi:10.1038/nbt.2199

designed to detect and cleave "loop-out" regions between hybrids of wild type promoter DNA sequences and DNA sequences in promoters containing TALEN-generated nucleotide deletions or insertions. If loop-out sequences are present, cleavage by the single-strand DNA-specific Surveyor nuclease should generate two DNA fragments, one in the range of 230 bp and the other in the range of 330 bp. Molecular sizes are indicated in base pairs at the left side of the ethidium bromide stained gel image. (c) DNA sequencing chromatograms of three DNA fragments derived from wild type (WT) tissue and two callus lines (#1, #2) each containing biallelic mutations (i.e., a deletion of 49 bp in one *OS11N3* allele and a 8 bp deletion in the other alleles of the *OS*11N23 gene in callus #1; a deletion of 6 bp in one allele in the promoter of *OS11N3* in callus #2 and a 5 bp deletion in the other allele).

Nature Biotechnology: doi:10.1038/nbt.2199

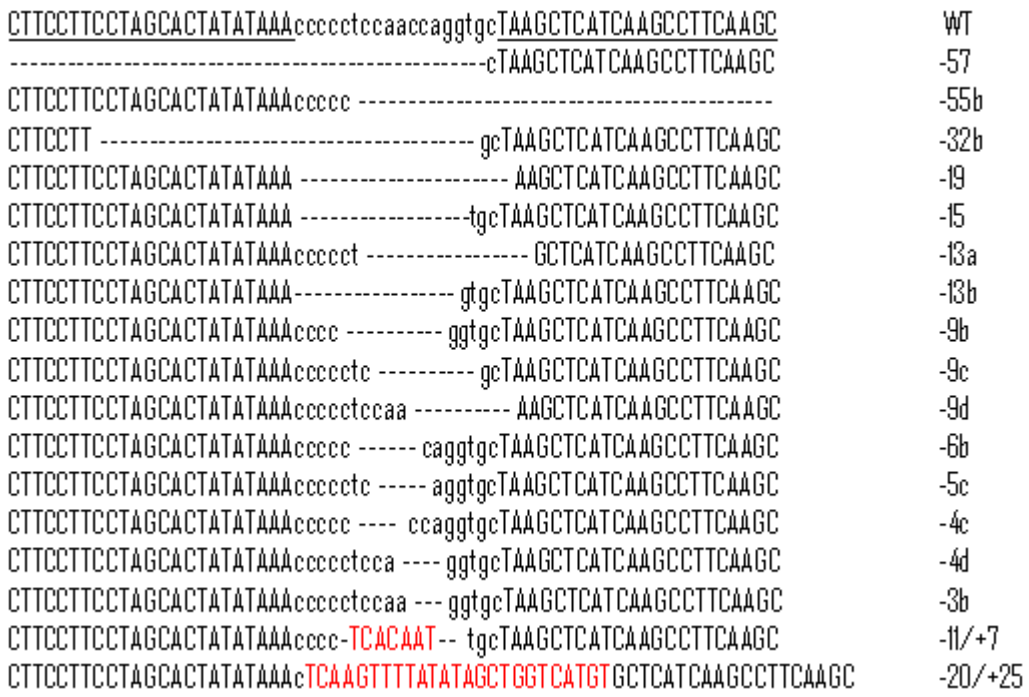
Supplementary Figure 4. Sequence of *Os11N3* gene mutations in T1 plants induced by the Pair 1 TALENs. Deletions and insertions are indicated by dashes and red letters, respectively. TALEN-binding sequences are underlined in the wild type (WT) gene sequence. Numbers and letters designating each individual mutant (with numbers reflecting the length of nucleotide deletions or insertions) are indicated to the right side of the DNA sequence.

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 5. Disease resistance in transgenic rice T1 plants. Lesion lengths caused by infection with a pathogenic AvrXa7-dependent Xoo strain were measured 14 days after inoculation of 20 T1 mutant plants (1 – 20) generated from TALEN pair 2 and 2 wild type Kitake plants (21 – 22). The T1 plants contained either homozygous monoallelic or heterozygous biallelic EBE mutations as confirmed by genotyping through sequencing. Leaf lesion lengths of 1~4 cm indicate disease resistance and lesion lengths of 10 ~14 cm indicate disease susceptibility. Error bars indicate 1 SD.

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 6. Additional haplotypes detected in T2 plants carrying *Os11N3* gene mutations produced with Pair 2 TALENs. Deletions and insertions are indicated by dashes and red letters, respectively. Numbers and letters designating each individual mutant (with numbers reflecting the length of nucleotide deletions or insertions) are indicated to the right side of the DNA sequence. TALEN-binding sequences are underlined in wild type (wt).

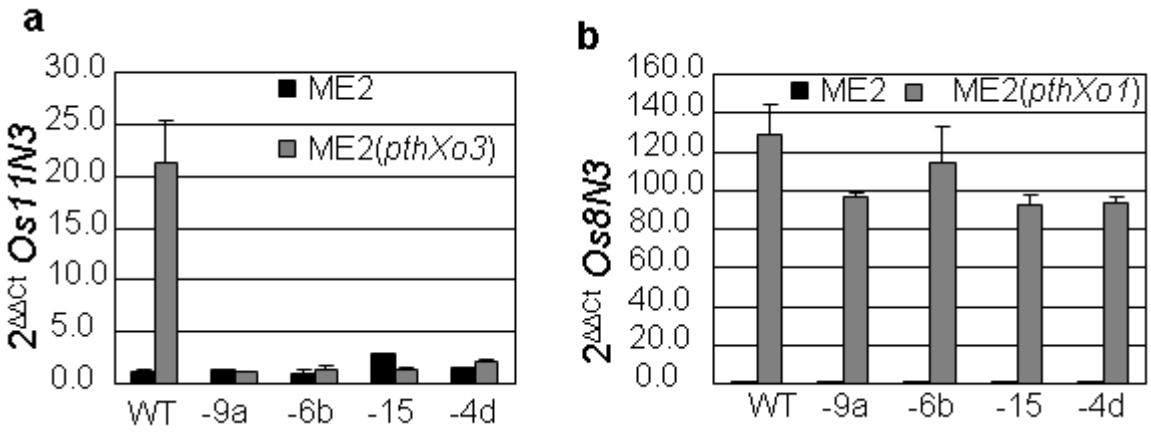
Nature Biotechnology: doi:10.1038/nbt.2199

	Genotype						
T1	-9a / -9a	-4d / -57			-9a / wt		
T2	-9a / -9a	-4d / -4d	-4d / -57	-57 / -57	-9a / -9a	-9a / wt	wt / wt
Ratio*	10/10	5/16	8/16	3/16	6/14	6/14	2/14

* Ratio denotes the number of T2 plants with the genotypes specified in T2 row to total number of T2 plants genotyped by sequencing the *Os11N3* promoter region.

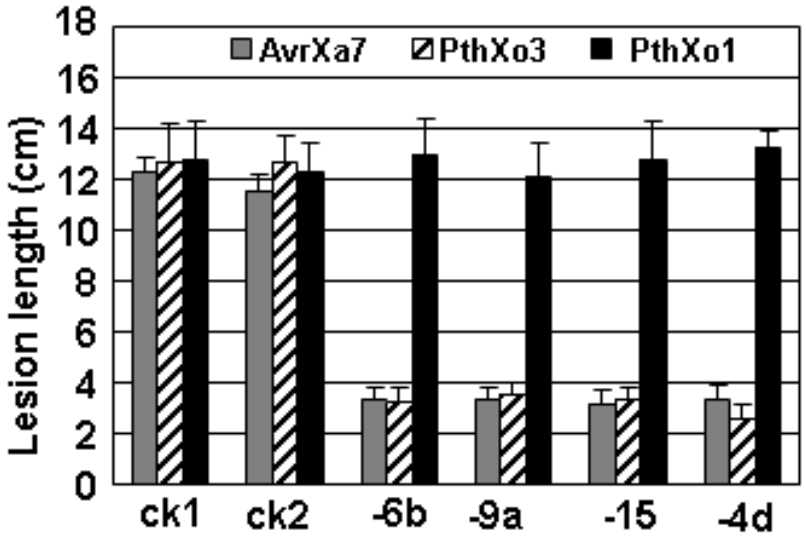
Supplementary Figure 7. Genetic Segregation of forty T2 plants derived from self-pollination of three T1 plants associated with the Pair 2 TALENs. Each of the two alleles of an individual plant are designated as being wild type (wt) or having a nucleotide deletion (-) and are separated top and bottom by a dividing line.

Nature Biotechnology: doi:10.1038/nbt.2199



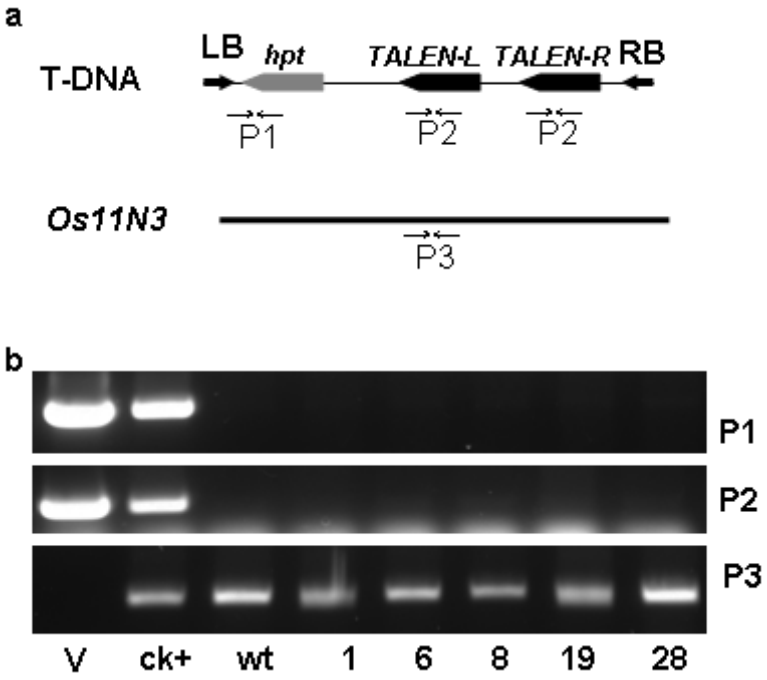
Supplementary Figure 8. Expression of *Os11N3* (**a**) and *Os8N3* (**b**), respectively, induced by PthXo3- and PthXo1-dependent Xoo strains in T2 plants of different genotypes (indicated below each column). Quantitative RT-PCR was performed with RNA derived from treatments of nonpathogenic Xoo strain ME2 and pathogenic Xoo strains ME2(*pthXo3*) and ME2(*pthXo1*). Transcript levels of the rice gene *OsTFIIAγ5* were used as a reference for measurements of *Os11N3* and *Os8N3* transcript levels.

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 9. Severity of disease damage to wild type and transgenic rice plants caused by AvrXa7-, PthXo3-, and PthXo1-dependent Xoo strains. Lengths of lesions in wild type plants (CK1), segregating T2 transgenic plants with intact *Os11N3* (CK2) and T2 transgenic plants homozygous for *Os11N3* promoter mutations of 6 bp (-6b), 9 bp (-9a), 15 bp (-15) and 4 bp (-4d) deletions, respectively, were measured 14 days post inoculation with different TAL effector Xoo strains as indicated.

Nature Biotechnology: doi:10.1038/nbt.2199



Supplementary Figure 10. Removal of T-DNA sequences containing TALEN genes from TALEN-modified rice plants using genetic crossing. (a) Schematic diagrams of the transfer DNA (T-DNA) region containing a paired set of TALENs and the *Os11N3* gene aligned with the paired primer sets P1, P2 and P3 used for specific gene segment detection by PCR amplification. LB and RB represent the left and right border sequences for *Agrobacterium*-mediated gene transfer; *hpt* represents the hygromycin resistance gene. (b) Gel images of PCR products obtained with the primer sets of P1, P2 and P3 for hygromycin phosphotransferase gene (*hpt*), TALEN genes and *Os11N3* promoter, respectively. Labels below gel images represent: v, binary vector DNA; ck+, a positive control of DNA from a transgenic plant containing the T-DNA region depicted in (a) and the *Os11N3* gene; wt, DNA from a nontransgenic, wild type rice plant; number, individual T1 plants selected from genetic crosses to lack the T-DNA region, but retain a functional *Os11N3* promoter region containing inactivated or deleted AvrXa7 and PthXo3 EBE sites.

Nature Biotechnology: doi:10.1038/nbt.2199

Supplementary Note

Open reading frame sequences of three designer TALENs

1. dTALEN-R1

ATGGATCCCATTCGTTCGCGCACGCCAAGTCCTGCCCGCGAGCTTCTGCCCGGACCCCAACCGGATAGGG
TTCAGCCGACTGCAGATCGGGGGGGGGCTCCGCCTGCTGGCGGCCCCCTGGATGGCTTGCCCGCTCGGCG
GACGATGTCCCGGACCCGGCTGCCATCTCCCCCTGCGCCCTCGCCTGCGTTCTCGGCGGGCAGCTTCAGC
GATCTGCTCCGTCAGTTCGATCCGTCGCTTCTTGATACATCGCTTCTTGATTCGATGCCTGCCGTCGGCA
CGCCGCATACAGCGGCTGCCCCAGCAGAGTGGGATGAGGTGCAATCGGGTCTGCGTGCAGCCGATGACCC
GCCACCCACCGTGCGTGTCGCTGTCACTGCCGCGCGGCCGCCGCGCGCCAAGCCGGCCCCGCGACGGCGT
GCGGCGCAACCCTCCGACGCTTCGCCGGCCGCGCAGGTGGATCTACGCACGCTCGGCTACAGTCAGCAGC
AGCAAGAGAAGATCAAACCGAAGGTGCGTTCGACAGTGGCGCAGCACCACGAGGCACTGGTGGGCCATGG
GTTTACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAGTAT
CAGCACATAATCACGGCGTTGCCAGAGGCGACACACGAAGACATCGTTGGCGTCGGCAAACAGTGGTCCG
GCGCACGCGCCCTGGAGGCCTTGCTCACGAAGGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACAC
AGGCCAACTTCTCAAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCATGGCGCAAT
GCACTGACGGGTGCCCCCCTGAACCTGACCCCGGCACAGGTGGTGGCCATCGCCAGCCACGATGGCGGCA
AGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCA
GGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCCTTGGAGACGGTGCAGCGGCTGTTGCCGGTG
CTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGG
CTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGT
GGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGC
CAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCTTTGG
AGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCAT
CGCCAGCAATATTGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGAC
CATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCCCTGGAGACGG
TACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAG
CAATGGCGGCGGCAAGCAGGCACTGGAAACACTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCCTTGGAGACGGTGCAGC
GGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGA
TGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACC
CCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGT
TGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGG
CAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGAC
CAGGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGG
TGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCA
GGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTC
GTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCactggagaCTGTACAGCGGCTGTTGCCGGTGCTGT
GCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCCTT
GGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGCCCAAGTGGTGGCC
ATCGCCAGCAATGGCGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGG
ACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGTTGGAGAC
GGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCC
AACAATAACGGCGGCAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATG
GCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCATTGGAGACGGTACA
GCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAAT
GGCGGCGGCAAGCAGGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGA
CCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCACTGGAGACGGTACAGCGGCT
GTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGAACCAGGTGGTGGCCATCGCCAGCAATGGCGGC
aAGCAGGCGCTGGAGAGCATTGTTGCCCAGTTATCTCGCCCTGATCCGGCGTTGGCCGCGTTGAcCAACG

Nature Biotechnology: doi:10.1038/nbt.2199

AcCACCTCGTCGCCTTGGCCTGCCTCGGCGGACGTCCTGCCCTGGATGCAGTGAAAAAGGGATTGCCGCA
CGCGCCGGAATTGATCAGAAGAATCAATCGCCGCATTCCCGAACGCACGTCCCATCGCGTTCCCGACCTC
GCGCACGTGGTTCGCGTGCTTGGTTTTTTCCAGAGCCACTCCCACCCAGCGCAAGCATTCGATGACGCCA
TGACGCAGTTCGAGATGAGCAGGCACGGCTTGGTACAGCTCTTTCGCAGAGTGGGCGTCACCGAATTCGA
AGCCCGCTACGGAACGCTCCCCCCAGCCTCGCAGCGTTGGGACCGTATCCTCCAGGCATCAGGGATGAAA
AGGGCCAAACCGTCCCCTACTTCAGCTCAAACACCGGATCAGGCGTCTTTGCATGCAGATTACAAGGACG
ACGACGACAAGAAGGATTACAAGGACGACGACGACAAGAAGGGTCGACCCAGCCCAATGCACGAGGGAGA
TCAGACGCGGGCAAGCAGCCGTAAACGGTCCCGATCGGATCGTGCTGTCACCGGCCCCTCCACACAGCAA
TCTTTCGAGGTGCGCGTTCCCGAACAGCAAGATGCGCTGCATTTGCCCCTCAGCTGGAGGGTAAAACGCC
CGCGTACCAGGATCGGGGGCGGCCTCCCGGATCCTGGTACGCCCATCGCTGCCGACCTGGCAGCGTCCAG
CACCGTGATGTGGGAACAAGATGCGGCCCCCTTCGCAGGGGCAGCGGATGATTTCCCGGCATTCAACGAA
GAGGAGCTCGCATGGTTGATGGAGCTATTGCCTCAGTCAGGCTCAGTCGGAGGGACGATCTCTAGACAGC
TAGTGAAATCTGAATTGGAAGAGAAGAAATCTGAACTTAGACATAAATTGAAATATGTGCCACATGAATA
TATTGAATTGATTGAAATCGCAAGAAATTCAACTCAGGATAGAATCCTTGAAATGAAGGTGATGGAGTTC
TTTATGAAGGTTTATGGTTATCGTGGTAAACATTTGGGTGGATCAAGGAAACCAGACGGAGCAATTTATA
CTGTCGGATCTCCTATTGATTACGGTGTGATCGTTGATACTAAGGCATATTCAGGAGGTTATAATCTTCC
AATTGGTCAAGCAGATGAAATGCAAAGATATGTCGAAGAGAATCAAACAAGAAACAAGCATATCAACCCT
AATGAATGGTGGAAAGTCTATCCATCTTCAGTAACAGAATTTAAGTTCTTGTTTGTGAGTGGTCATTTCA
AAGGAAACTACAAAGCTCAGCTTACAAGATTGAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGT
AGAAGAGCTTTTGATTGGTGGAGAAATGATTAAAGCTGGTACATTGACACTTGAGGAAGTGAGAAGGAAA
TTTAATAACGGCGAGATAAACTTTTAA

2. dTALEN-L2

ATGGATCCCATTCGTTCGCGCACGCCAAGTCCTGCCCGCGAGCTTCTGCCCGGACCCCAACCGGATAGGG
TTCAGCCGACTGCAGATCGGGGGGGGGCTCCGCCTGCTGGCGGCCCCCTGGATGGCTTGCCCGCTCGGCG
GACGATGTCCCGGACCCGGCTGCCATCTCCCCCTGCGCCCTCGCCTGCGTTCTCGGCGGGCAGCTTCAGC
GATCTGCTCCGTCAGTTCGATCCGTCGCTTCTTGATACATCGCTTCTTGATTCGATGCCTGCCGTCGGCA
CGCCGCATACAGCGGCTGCCCCAGCAGAGTGGGATGAGGTGCAATCGGGTCTGCGTGCAGCCGATGACCC
GCCACCCACCGTGCGTGTCGCTGTCACTGCCGCGCGGCCGCCGCGCGCCAAGCCGGCCCCGCGACGGCGT
GCGGCGCAACCCTCCGACGCTTCGCCGGCCGCGCAGGTGGATCTACGCACGCTCGGCTACAGTCAGCAGC
AGCAAGAGAAGATCAAACCGAAGGTGCGTTCGACAGTGGCGCAGCACCACGAGGCACTGGTGGGCCATGG
GTTTACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAGTAT
CAGCACATAATCACGGCGTTGCCAGAGGCGACACACGAAGACATCGTTGGCGTCGGCAAACAGTGGTCCG
GCGCACGCGCCCTGGAGGCCTTGCTCACGAAGGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACAC
AGGCCAACTTCTCAAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCATCGCGCAAT
GCACTGACGGGTGCCCCCCTGAACCTGACCCCGGCACAGGTGGTGGCCATCGCCAGCCACGATGGCGGCA
AGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCA
GGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCCTTGGAGACGGTGCAGCGGCTGTTGCCGGTG
CTGTGCCAGGCCCATGGCCTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGG
CTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCAGGACCAGGTGGT
GGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGC
CAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCTTTGG
AGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCAT
CGCCAGCAATGGCGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGAC
CATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCCCTGGAGACGG
TACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCAGGACCAGGTGGTGGCCATCGCCAG
CCACGATGGCGGCAAGCAGGCACTGGAAACACTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCCTTGGAGACGGTGCAGC
GGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGG
CGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACC
CCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGT

Nature Biotechnology: doi:10.1038/nbt.2199

TGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAACAATAACGGCGG
CAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGGCCATGGCCTGACCCAGGAC
CAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGG
TGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCA
GGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTG
GTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCACTGGAGACTGTACAGCGGCTGTTGCCGGTGCTGT
GCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCCTT
GGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCC
ATCGCCAGCAATATTGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGG
ACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGTTGGAGAC
GGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCC
AGCAATATTGGCGGCAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATG
GCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCATTGGAGACGGTACA
GCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAGCAAT
ATTGGCGGCAAGCAGGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGA
CCCCGGACCAGGTGGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCACTGGAGACGATTGTTGCCCA
GTTATCTCGCCCTGATCCGGCGTTGGCCGCGTTGACCAACGACCACCTCGTCGCCTTGGCCTGCCTCGGC
GGACGTCCTGCCCTGGATGCAGTGAAAAAGGGATTGCCGCACGCGCCGGAATTGATCAGAAGAATCAATC
GCCGCATTCCCGAACGCACGTCCCATCGCGTTCCCGACCTCGCGCACGTGGTTCGCGTGCTTGGTTTTTT
CCAGAGCCACTCCCACCCAGCGCAAGCATTCGATGACGCCATGACGCAGTTCGAGATGAGCAGGCACGGC
TTGGTACAGCTCTTTCGCAGAGTGGGCGTCACCGAATTCGAAGCCCGCTACGGAACGCTCCCCCCAGCCT
CGCAGCGTTGGGACCGTATCCTCCAGGCATCAGGGATGAAAAGGGCCAAACCGTCCCCTACTTCAGCTCA
AACACCGGATCAGGCGTCTTTGCATGCAGATTACAAGGACGACGACGACAAGAAGGATTACAAGGACGAC
GACGACAAGAAGGGTCGACCCAGCCCAATGCACGAGGGAGATCAGACAGGGGCAAGCAGCCGTAAACGGT
CCCGATCGGATCGTGCTGTCACCGGCCCCTCCGCACAGCAATCTTTCGAGGTGCGCGTTCCCGAACAGCG
CGATGCGCTGCATTTGCCCCTCAGCTGGAGGGTAAAACGCCCGCGTACCAGGATCGGGGGCGGCCTCCCG
GATCCTGGTACGCCCATCGCTGCCGACCTGGCAGCGTCCAGCACCGTGATCagatccCAGCTAGTGAAAT
CTGAATTGGAAGAGAAGAAATCTGAACTTAGACATAAATTGAAATATGTGCCACATGAATATATTGAATT
GATTGAAATCGCAAGAAATTCAACTCAGGATAGAATCCTTGAAATGAAGGTGATGGAGTTCTTTATGAAG
GTTTATGGTTATCGTGGTAAACATTTGGGTGGATCAAGGAAACCAGACGGAGCAATTTATACTGTCGGAT
CTCCTATTGATTACGGTGTGATCGTTGATACTAAGGCATATTCAGGAGGTTATAATCTTCCAATTGGTCA
AGCAGATGAAATGCAAAGATATGTCGAAGAGAATCAAACAAGAAACAAGCATATCAACCCTAATGAATGG
TGGAAAGTCTATCCATCTTCAGTAACAGAATTTAAGTTCTTGTTTGTGAGTGGTCATTTCAAAGGAAACT
ACAAAGCTCAGCTTACAAGATTGAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCT
TTTGATTGGTGGAGAAATGATTAAAGCTGGTACATTGACACTTGAGGAAGTGAGAAGGAAATTTAATAAC
GGTGAGATAAACTTTTAA

3. dTALEN-R2

ATGGATCCCATTCGTTCGCGCACGCCAAGTCCTGCCCGCGAGCTTCTGCCCGGACCCCAACCGGATAGGG
TTCAGCCGACTGCAGATCGGGGGGGGGCTCCGCCTGCTGGCGGCCCCCTGGATGGCTTGCCCGCTCGGCG
GACGATGTCCCGGACCCGGCTGCCATCTCCCCCTGCGCCCTCGCCTGCGTTCTCGGCGGGCAGCTTCAGC
GATCTGCTCCGTCAGTTCGATCCGTCGCTTCTTGATACATCGCTTCTTGATTCGATGCCTGCCGTCGGCA
CGCCGCATACAGCGGCTGCCCCAGCAGAGTGGGATGAGGTGCAATCGGGTCTGCGTGCAGCCGATGACCC
GCCACCCACCGTGCGTGTCGCTGTCACTGCCGCGCGGCCGCCGCGCGCCAAGCCGGCCCCGCGACGGCGT
GCGGCGCAACCCTCCGACGCTTCGCCGGCCGCGCAGGTGGATCTACGCACGCTCGGCTACAGTCAGCAGC
AGCAAGAGAAGATCAAACCGAAGGTGCGTTCGACAGTGGCGCAGCACCACGAGGCACTGGTGGGCCATGG
GTTTACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAGTAT
CAGCACATAATCACGGCGTTGCCAGAGGCGACACACGAAGACATCGTTGGCGTCGGCAAACAGTGGTCCG
GCGCACGCGCCCTGGAGGCCTTGCTCACGAAGGCGGGGGAGTTGAGAGGTCCGCCGTTACAGTTGGACAC
AGGCCAACTTCTCAAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCATCGCGCAAT
GCACTGACGGGTGCCCCCCTGAACCTGACCCCGGCACAGGTGGTGGCCATCGCCAACAATAACGGCGGCA

Nature Biotechnology: doi:10.1038/nbt.2199

AGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCAGGACCA
GGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCCTTGGAGACGGTGCAGCGGCTGTTGCCGGTG
CTGTGCCAGGCCCATGGCCTGACCCCGGCCCAAGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGG
CTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGT
GGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGC
CAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCTTTGG
AGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCCAT
CGCCAGCAATATTGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGAC
CATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCCCTGGAGACGG
TACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAA
CAATAACGGCGGCAAGCAGGCACTGGAAACACTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGC
CTGACCCCGGACCAGGTCGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCCTTGGAGACGGTGCAGC
GGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAAGTCGTGGCCATCGCCAGCCACGA
TGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACC
CCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGTTGGAGACGGTACAGCGGCTGT
TGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATGGCGGCGG
CAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGAC
CAGGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCATTGGAGACGGTACAGCGGCTGTTGCCGG
TGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCA
GGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTC
GTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCACTGGAGACTGTACAGCGGCTGTTGCCGGTGCTGT
GCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCCTT
GGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTGGTGGCC
ATCGCCAGCAATATTGGCGGCAAGCAGGCTCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGG
ACCATGGCCTGACCCCGGACCAGGTGGTGGCCATCGCCAACAATAACGGCGGCAAGCAGGCGTTGGAGAC
GGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCAGGACCAGGTGGTGGCCATCGCC
AGCCACGATGGCGGCAAGCAGGCTTTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATG
GCCTGACCCCGGACCAGGTCGTGGCCACCGCCAGCAATGGCGGCGGCAAGCAGGCATTGGAGACGGTACA
GCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGACCCCGGACCAGGTCGTGGCCATCGCCAGCAAT
GGCGGCGGCAAGCAGGCCCTGGAGACGGTACAGCGGCTGTTGCCGGTGCTGTGCCAGGACCATGGCCTGA
CCCCGGACCAGGTCGTGGCCATCGCCAGCAATATTGGCGGCAAGCAGGCACTGGAGACGATTGTTGCCCA
GTTATCTCGCCCTGATCCGGCGTTGGCCGCGTTGACCAACGACCACCTCGTCGCCTTGGCCTGCCTCGGC
GGACGTCCTGCCCTGGATGCAGTGAAAAAGGGATTGCCGCACGCGCCGGAATTGATCAGAAGAATCAATC
GCCGCATTCCCGAACGCACGTCCCATCGCGTTCCCGACCTCGCGCACGTGGTTCGCGTGCTTGGTTTTTT
CCAGAGCCACTCCCACCCAGCGCAAGCATTCGATGACGCCATGACGCAGTTCGAGATGAGCAGGCACGGC
TTGGTACAGCTCTTTCGCAGAGTGGGCGTCACCGAATTCGAAGCCCGCTACGGAACGCTCCCCCCAGCCT
CGCAGCGTTGGGACCGTATCCTCCAGGCATCAGGGATGAAAAGGGCCAAACCGTCCCCTACTTCAGCTCA
AACACCGGATCAGGCGTCTTTGCATGCAGATTACAAGGACGACGACGACAAGAAGGATTACAAGGACGAC
GACGACAAGAAGGGTCGACCCAGCCCAATGCACGAGGGAGATCAGACGCGGGCAAGCAGCCGTAAACGGT
CCCGATCGGATCGTGCTGTCACCGGCCCCTCCACACAGCAATCTTTCGAGGTGCGCGTTCCCGAACAGCA
AGATGCGCTGCATTTGCCCCTCAGCTGGAGGGTAAAACGCCCGCGTACCAGGATCGGGGGCGGCCTCCCG
GATCCTGGTACGCCCATCGCTGCCGACCTGGCAGCGTCCAGCACCGTGATGTGGGAACAAGATGCGGCCC
CCTTCGCAGGGGCAGCGGATGATTTCCCGGCATTCAACGAAGAGGAGCTCGCATGGTTGATGGAGCTATT
GCCTCAGTCAGGCTCAGTCGGAGGGACGATCTCTAGACAGCTAGTGAAATCTGAATTGGAAGAGAAGAAA
TCTGAACTTAGACATAAATTGAAATATGTGCCACATGAATATATTGAATTGATTGAAATCGCAAGAAATT
CAACTCAGGATAGAATCCTTGAAATGAAGGTGATGGAGTTCTTTATGAAGGTTTATGGTTATCGTGGTAA
ACATTTGGGTGGATCAAGGAAACCAGACGGAGCAATTTATACTGTCGGATCTCCTATTGATTACGGTGTG
ATCGTTGATACTAAGGCATATTCAGGAGGTTATAATCTTCCAATTGGTCAAGCAGATGAAATGCAAAGAT
ATGTCGAAGAGAATCAAACAAGAAACAAGCATATCAACCCTAATGAATGGTGGAAAGTCTATCCATCTTC
AGTAACAGAATTTAAGTTCTTGTTTGTGAGTGGTCATTTCAAAGGAAACTACAAAGCTCAGCTTACAAGA
TTGAATCATATCACTAATTGTAATGGAGCTGTTCTTAGTGTAGAAGAGCTTTTGATTGGTGGAGAAATGA
TTAAAGCTGGTACATTGACACTTGAGGAAGTGAGAAGGAAATTTAATAACGGCGAGATAAACTTTTAA

Nature Biotechnology: doi:10.1038/nbt.2199

References

1. Li, T. *et al. Nucleic Acids Res.* **39**, 359-372 (2011).
2. Li, T. *et al. Nucleic Acids Res.***39**,6315-6325 (2011).
3. Christensen, A. H., Quail, P. H. *Transgenic Res.* **5**, 213-218 (1996).
4. Odell, J. T., Nagy, F., Chua, N. H. *Nature* **313**, 810-812 (1985).
5. Hiei, Y. *et al. Plant J.* **6**, 271 (1994).
6. Edwards, K., Johnstone, C., Thompson, C. *Nucleic Acids Res.* **19**, 1349 (1991).
7. Yang, B., Sugio, A., White, F.F. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 10503-10508 (2006).
8. Livak, K.J., Schmittgen, T.D. *Methods* **25,** 402-408 (2001)
9. Kauffman, H. E. *et al*. *Plant Dis. Rep.* **57**, 537-541 (1973).

Nature Biotechnology: doi:10.1038/nbt.2199