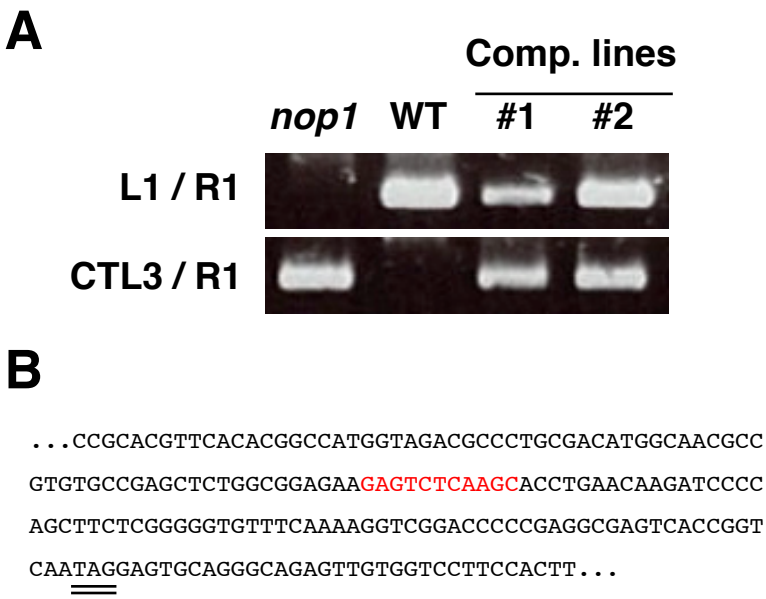
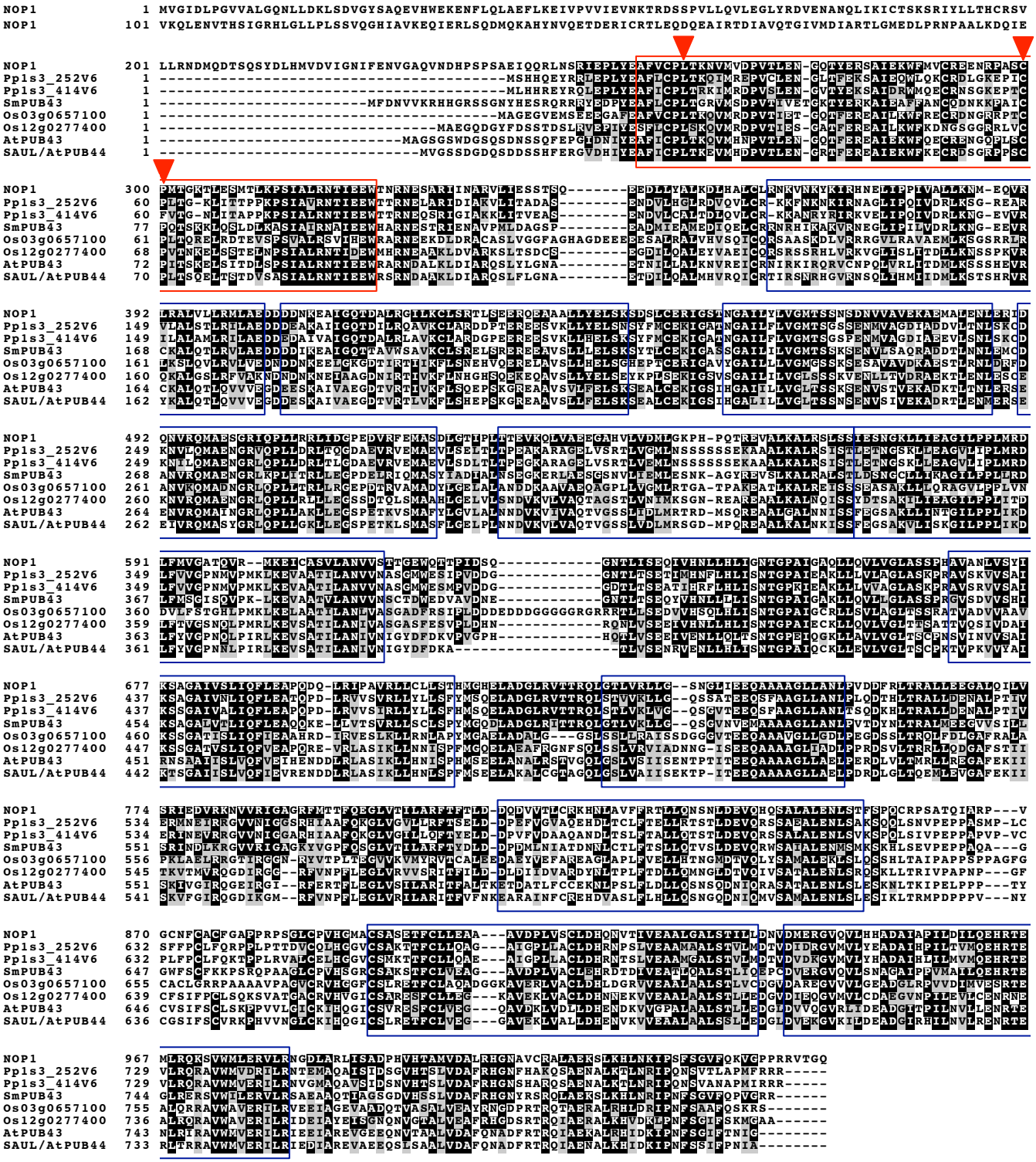


Supplemental Figure 1. Molecular Characterization of the T-DNA Insertion Site of *nop1*.

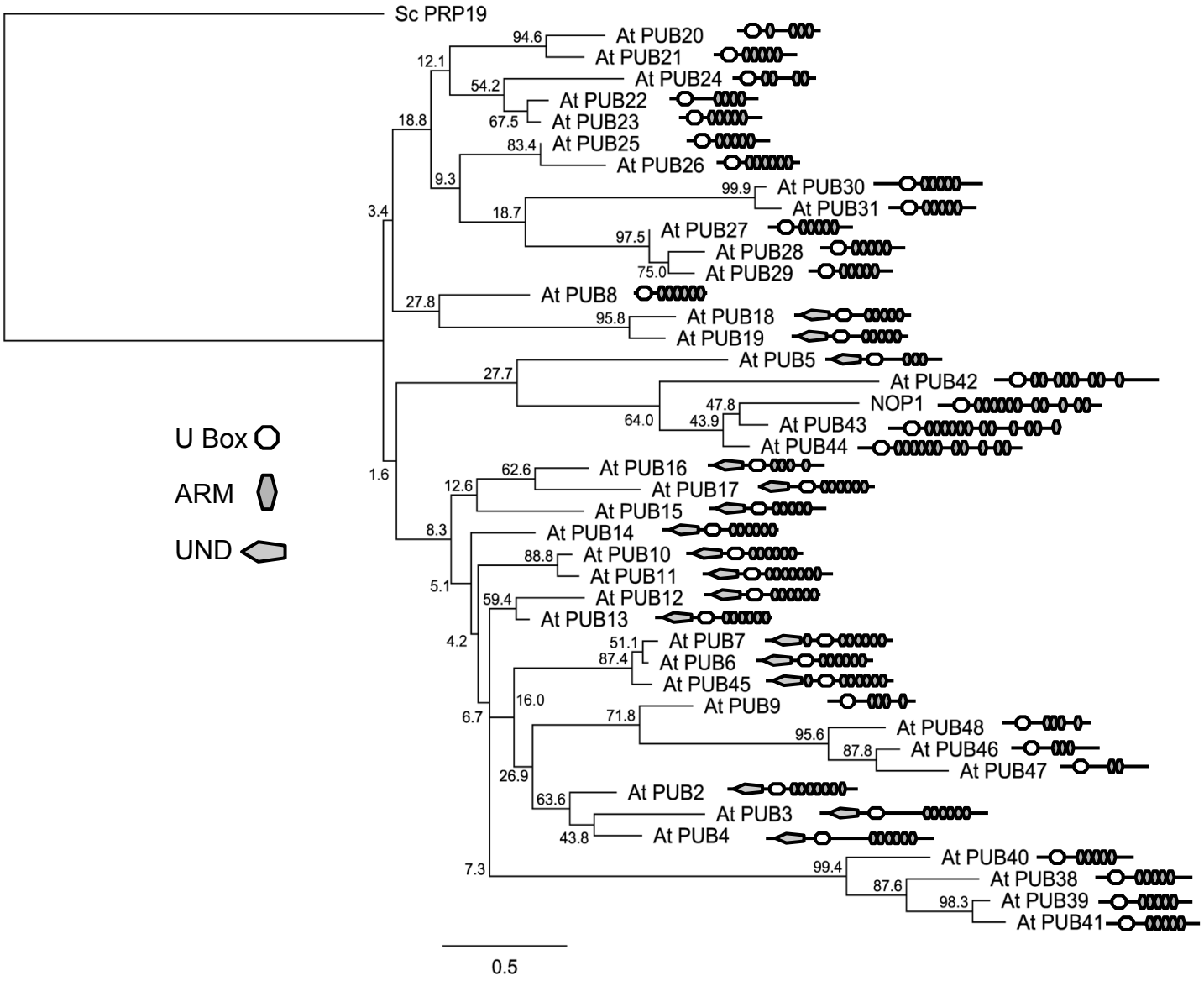
(A) Genomic PCR analyses of wild type, *nop1*, and the complemented lines, with the primer sets indicated on the left. The positions of the primers in the *NOP1* genomic locus are represented in Figure 4A.

(B) Sequence of the T-DNA insertion site in *NOP1* gene. The 11-bp sequence indicated in red near the stop codon in *NOP1* coding region was deleted and replaced with T-DNA in *nop1*. Double underline shows the stop codon of *NOP1* gene.

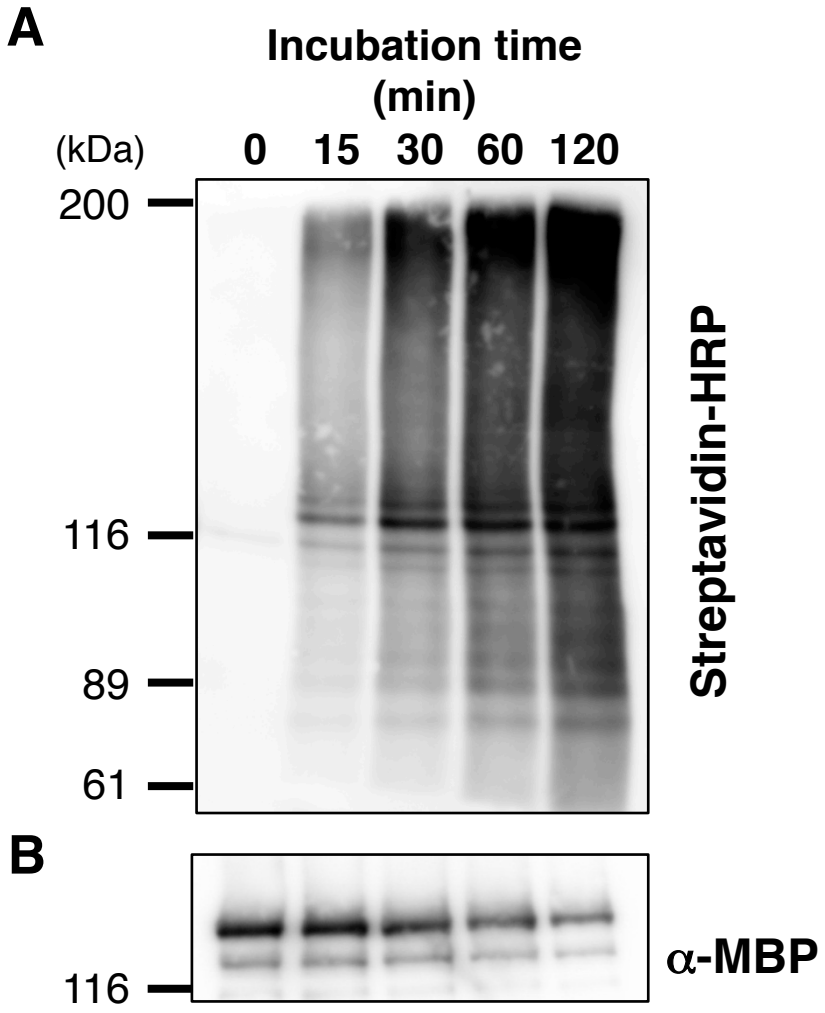


Supplemental Figure 2. Amino Acid Sequence Alignment of NOP1 and Seven Other PUB-ARM Proteins.

Red box indicates U-box domain. Blue boxes indicate armadillo repeats detected using SMART, a simple modular architecture research tool (http://smart.embl-heidelberg.de). Residues identical or similar to those of NOP1 are shown in black or gray shading, respectively. Red arrow heads indicate amino acid residues mutated in NOP1 E3 ligase activity assay shown in Figure 5.

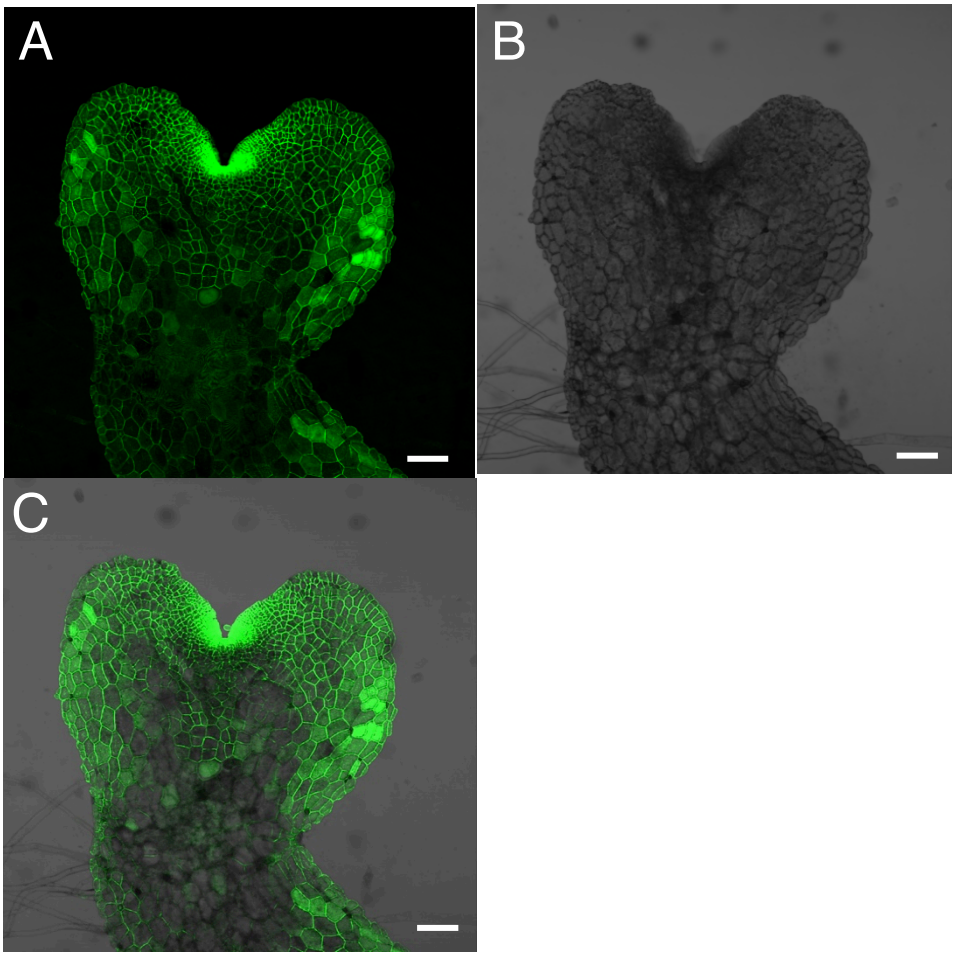


Supplemental Figure 3. Phylogeny of NOP1 and the Arabidopsis PUB-ARM proteins.
The tree was constructed with phyML (Guindon and Gascuel, 2000) (https://code.google.com/p/phyml/) using maximum-likelihood principle, on the basis of the multiple alignment of the U-box domains (see Supplemental Data Set 1 online). The U-box domains of NOP1, the 41 Arabidopsis PUB-ARM proteins, and PRP19 (YLL036C) from *Saccharomyces cerevisiae* were analyzed. The 41 Arabidopsis PUB-ARM proteins are as follows: At5g67340 (At PUB2), At3g54790 (At PUB3), At2g23140 (At PUB4), At4g36550 (At PUB5), At1g24330 (At PUB6), At1g67530 (At PUB7), At4g21350 (At PUB8), At3g07360 (At PUB9), At1g71020 (At PUB10), At1g23030 (At PUB11), At2g28830 (At PUB12), At3g46510 (At PUB13), At3g54850 (At PUB14), At5g42340 (At PUB15), At5g01830 (At PUB16), At1g29340 (At PUB17), At1g10560 (At PUB18), At1g60190 (At PUB19), At1g66160 (At PUB20), At5g37490 (At PUB21), At3g52450 (At PUB22), At2g35930 (At PUB23), At3g11840 (At PUB24), At3g19380 (At PUB25), At1g49780 (At PUB26), At5g64660 (At PUB27), At5g09800 (At PUB28), At3g18710 (At PUB29), At3g49810 (At PUB30), At5g65920 (At PUB31), At5g65200 (At PUB38), At3g47820 (At PUB39), At5g40140 (At PUB40), At5g62560 (At PUB41), At1g68940 (At PUB42), At1g76390 (At PUB43), At1g20780 (At PUB44), At1g27910 (At PUB45), At5g18320 (A tPUB46), At5g18330 (At PUB47), At5g18340 (At PUB48). Number of bootstrap trials is 2000. Bootstrap values of key nodes are shown at respective nodes as percentages. Scale bar indicates number of amino acid changes per branch length.



Supplemental Figure 4. Time-Dependent Assay of an *In Vitro* Ubiquitination of NOP1 Protein.

MBP-NOP1 was incubated with E1, E2, biotin-Ub, and ATP for 0, 15, 30, 60, and 120 min and then subjected to Western blot analysis with streptavidin-HRP (top) or anti-MBP antibodies (bottom).



Supplemental Figure 5. Expression of *NOP1* Gene in Developing Thallus.
(A-C) Z-stack images of optical serial sections. Optical serial sections through 3-day-old *gNOP1-Citrine/nop1* #1 thallus. Sections were taken from ventral to dorsal region in 0.5 µm slices. Panels show Citrine fluorescence **(A)**, bright-field **(B)** and merged images **(C)**. Scale bars : 100µm.

Supplemental Table 1. Cycle Settings Used for TAIL-PCR

Reaction	Cycle no.	Thermal settings
Primary	1	94ºC, 30 sec, 95ºC, 15 sec
	5	94ºC, 15 sec, 65ºC, 30 sec, 68ºC, 3 min
	2	94ºC, 15 sec, 30ºC, 1 min, (3 min ramping to 68ºC), 68ºC, 3 min
	15	94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 44ºC, 30 sec, 68ºC, 3 min
	1	68ºC, 5 min
Secondary	1	94ºC, 30 sec, 95ºC, 30 sec
	10	94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 44ºC, 30 sec, 68ºC, 3 min
	1	68ºC, 5 min
Tertiary	1	94ºC, 30 sec, 95ºC, 30 sec
	10	94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 64ºC, 30 sec, 68ºC, 3 min
		94ºC, 15 sec, 44ºC, 30 sec, 68ºC, 3 min
	1	68ºC, 5 min

Supplemental Table 2. Primers Used in This Study

Name	Sequence (5'→3')	Usage
CTL1	GGTTCCTATAGGGTTTCGCTCAT	TAIL-PCR for the left border of pCAMBIA1300
CTL2	TGTTGAGCATATAAGAAACCCTTAG	TAIL-PCR for the left border of pCAMBIA1300
CTL3	AAAATCCAGTACTAAAATCCAGATCC	TAIL-PCR for the left border of pCAMBIA1300
CTR1	CTGGCGTAATAGCGAAGAGG	TAIL-PCR for the right border of pCAMBIA1300
CTR2	GATCGCCCTTCCCAACAG	TAIL-PCR for the right border of pCAMBIA1300
CTR3	CAGCCTGAATGGCGAATGCTA	TAIL-PCR for the right border of pCAMBIA1300
AD1	NTCGA(G/C)T(A/T)T(G/C)G(A/T)GTT	arbitrary degenerate primer for TAIL-PCR
AD2	NGTCGA(G/C)(A/T)GANA(A/T)GAA	arbitrary degenerate primer for TAIL-PCR
AD3	(A/T)GTGNAG (A/T)ANCANAGA	arbitrary degenerate primer for TAIL-PCR
PRO-L	CACCTTCTCGGAAGGTCGCTAAATC	Construction of entry clone containing NOP1 primer region
PRO-R2	AATATTACCAATCACATCGACCATGT	Construction of entry clone containing NOP1 primer region
gCDSL	CACCGAGCGGACTGGTCTCTTAGACTTA	Construction of entry clone containing NOP1 coding region
gCDSR-ns	TTGACCGGTGACTCGC	Construction of entry clone containing NOP1 coding region
L1	TCATCAAAACGTGACCATCG	Check of expression and T-DNA insertion in NOP1
R1	CACTTTCACCAGGCTTCGTC	Check of expression and T-DNA insertion in NOP1
MpEF1-L	TCACTCTGGGTGTGAAGCAG	Control of RT-PCR
MpEF1-R	GCCTCGAGTAAAGCTTCGTG	Control of RT-PCR
CDS-MBP-L	AAGGATTTCAGAATTCGGAGGCAGCGGTGGGTCCATGGTCGAT	Cloning of NOP1 CDS into pMAL-c2 vector
CDS-MBP-R	TTGCCTGCAGGTCGACTATTGACCGGTGACTCGCC	Cloning of NOP1 CDS into pMAL-c2 vector
L45S-F	GTACGAGGCGTTCGTTTGCCCATCAACAAAAAATGTGATGG	For introduction of a point mutation in the NOP1 CDS
L45S-R	CCATCACATTTTTTGTTGATGGGCAAACGAACGCCTCGTAC	For introduction of a point mutation in the NOP1 CDS
C83S-F	GGAGAACAGGCCTGCAAGTAGCCCCATGACAGGGAAG	For introduction of a point mutation in the NOP1 CDS
C83S-R	CTTCCCTGTCATGGGGCTACTTGCAGGCCTGTTCTCC	For introduction of a point mutation in the NOP1 CDS
P84A-F	CAGGCCTGCAAGTTGCGCCATGACAGGGAAGACTTTGG	For introduction of a point mutation in the NOP1 CDS
P84A-R	CCAAAGTCTTCCCTGTCATGGCGCAACTTGCAGGCCTG	For introduction of a point mutation in the NOP1 CDS

Underlined bases indicate the homologous sequences for cloning into pMAL-c2 using In-fusion cloning kit.