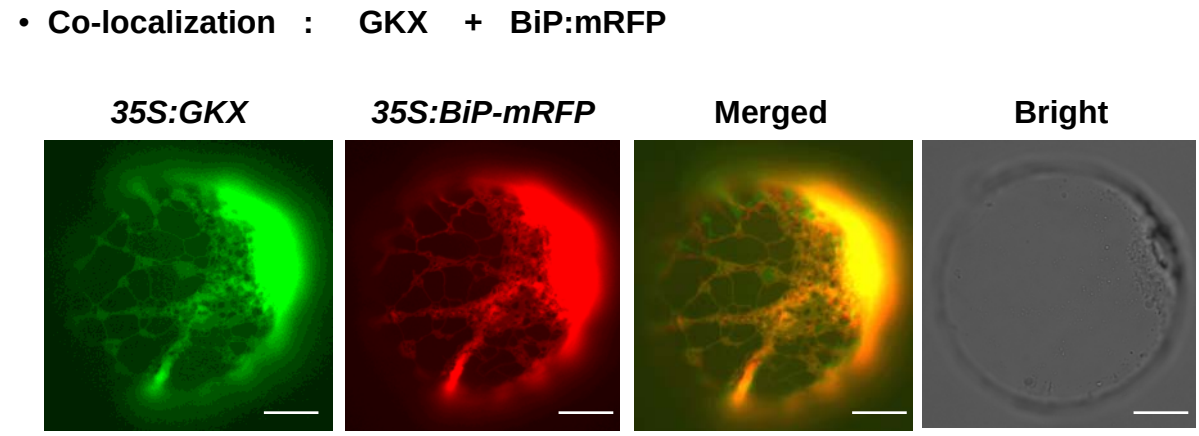
Supplemental data. Lee et al. (2009). Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic *Arabidopsis* plants



Supplemental Figure 1. Localization of GXK to the ER. Protoplasts were cotransformed with *GKX* and *BiP-mRFP*, and localization of these proteins was examined. Green fluorescent signals of GKX closely overlapped with the red fluorescent signals of BiP:mRFP in the network patterns, indicating that GKX localizes to the ER. Bars, 20 μm.

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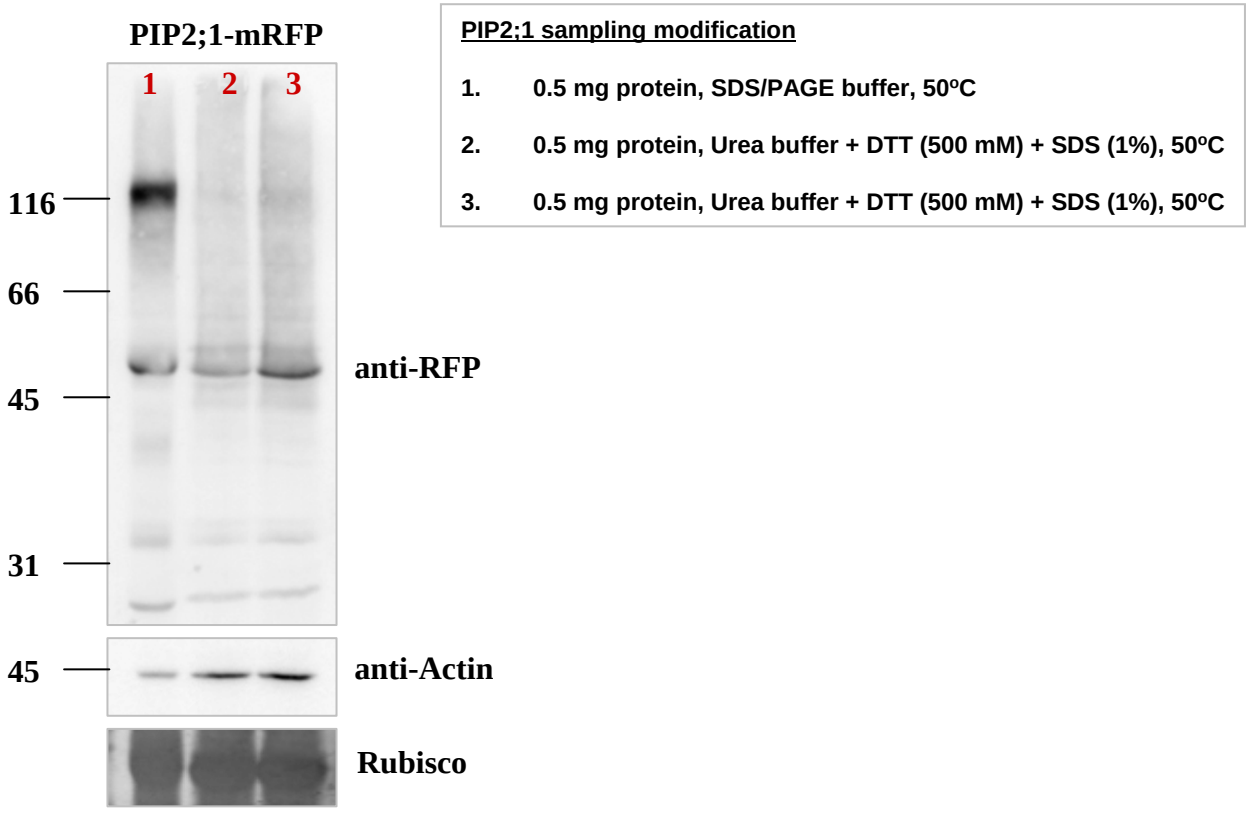

Supplemental Figure 2. Solubilization of PIP2;1-mRFP by urea-containing denaturation buffer. Protein extracts from protoplasts transformed with *PIP2;1-mRFP* was treated with regular SDS-PAGE buffer at 50°C or urea buffer (8 M urea, 50 mM Tris, pH 7.5, 250 mM sucrose, 1 mM $MgCl_2$, 1 mM DTT and 3% Triton X-100) supplemented with 500 mM DTT and 1% SDS for 30 min at 50°C and separated by SDS-PAGE. The blot was immunostained with anti-mRFP antibody. Actin and RbcL detected by anti-actin antibody and Coomassie blue staining, respectively, were used as loading controls.

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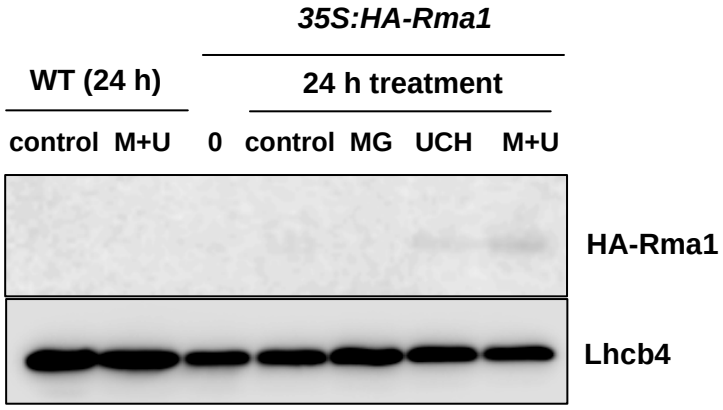

Supplemental Figure 3. Expression of HA-Rma1 protein in *35S:HA-Rma1 Arabidopsis* transgenic plants. The *35S:HA-Rma1 Arabidopsis* seedlings were incubated in the absence or presence of MG132 (25 μM), UCH-L3 (10 μM**),** or MG132 (25 μM) + UCH-L3 (10 μM**)**, for 24 h, and then protein extracts were analyzed by immuno-blotting using anti-HA or anti-Lhcb4 antibody.

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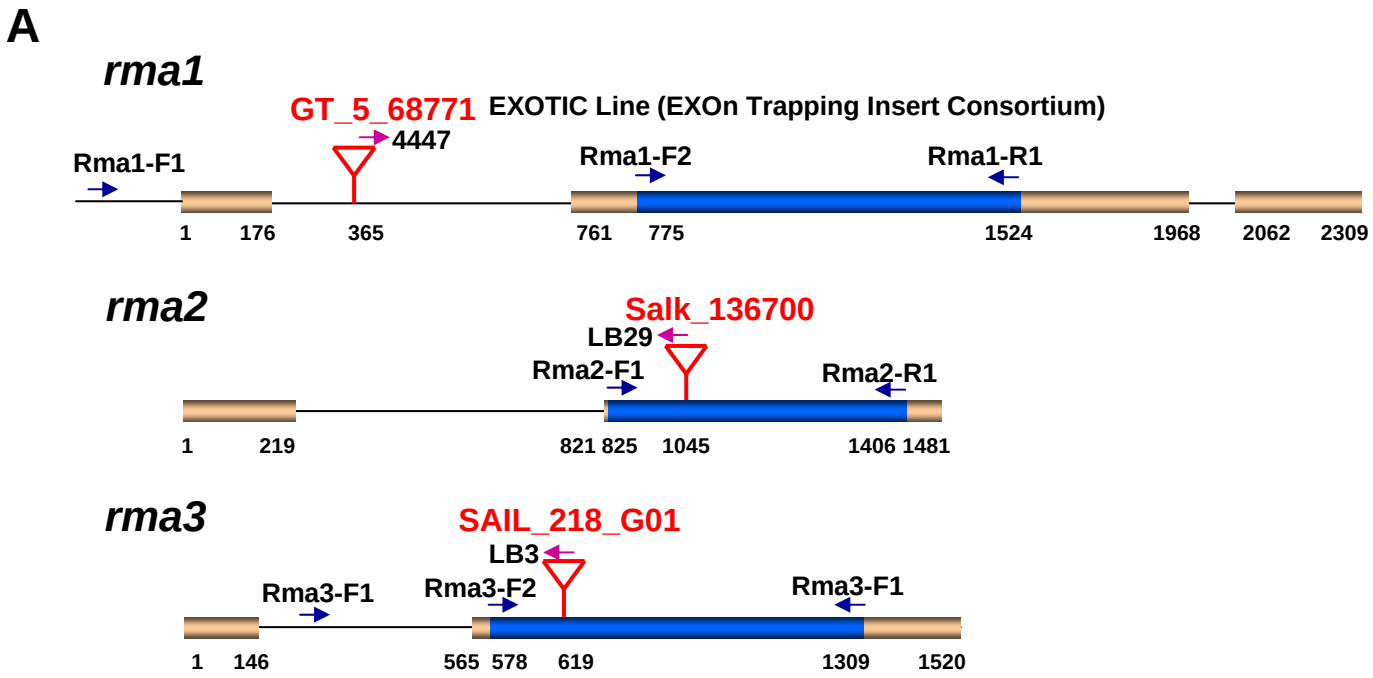

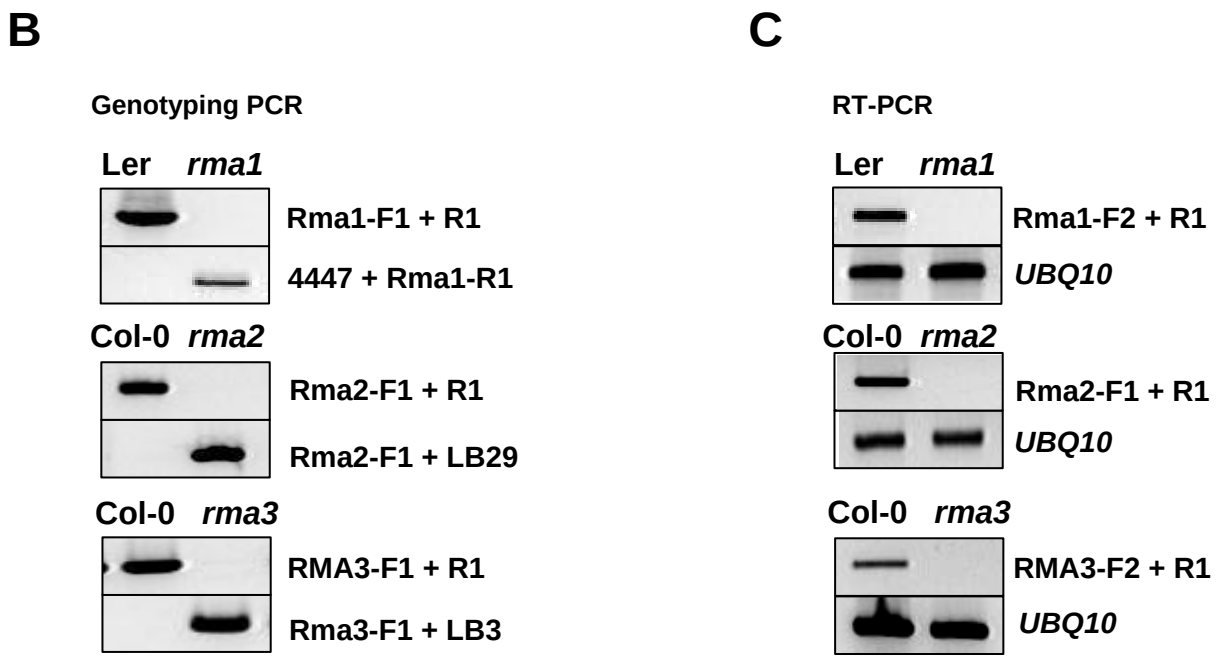

Supplemental Figure 4. Molecular characterization of the *rma1, rma2* and *rma3* mutant lines. (A) Schematic representation of the *rma1*, *rma2* and *rma3* alleles with the T-DNA insertion. The triangle represents the T-DNA. Blue bars indicate coding regions, while yellow bars show the 5'- and 3'-untranslated regions. Solid lines show introns. Gene-specific (forward and reverse) and T-DNA-specific (primer 4447, LB29 and LB3) primers used in the genotyping and RT-PCR are shown with arrows. **(B)** Genotyping of the *rma1, rma22* and *rma3* mutant plants. A set of gene-specific and T-DNA-specific primers used for genomic PCR are indicated in the right panel of agarose gel. **(C)** RT-PCR analysis of *Rma1*, *Rma2*, *Rma3* and *Ubiquitin10* mRNAs in wild type, *rma1*, *rma2*, and *rma3* plants. Primers used in genotyping PCR and RT-PCR are listed in Supplemental Table 1.

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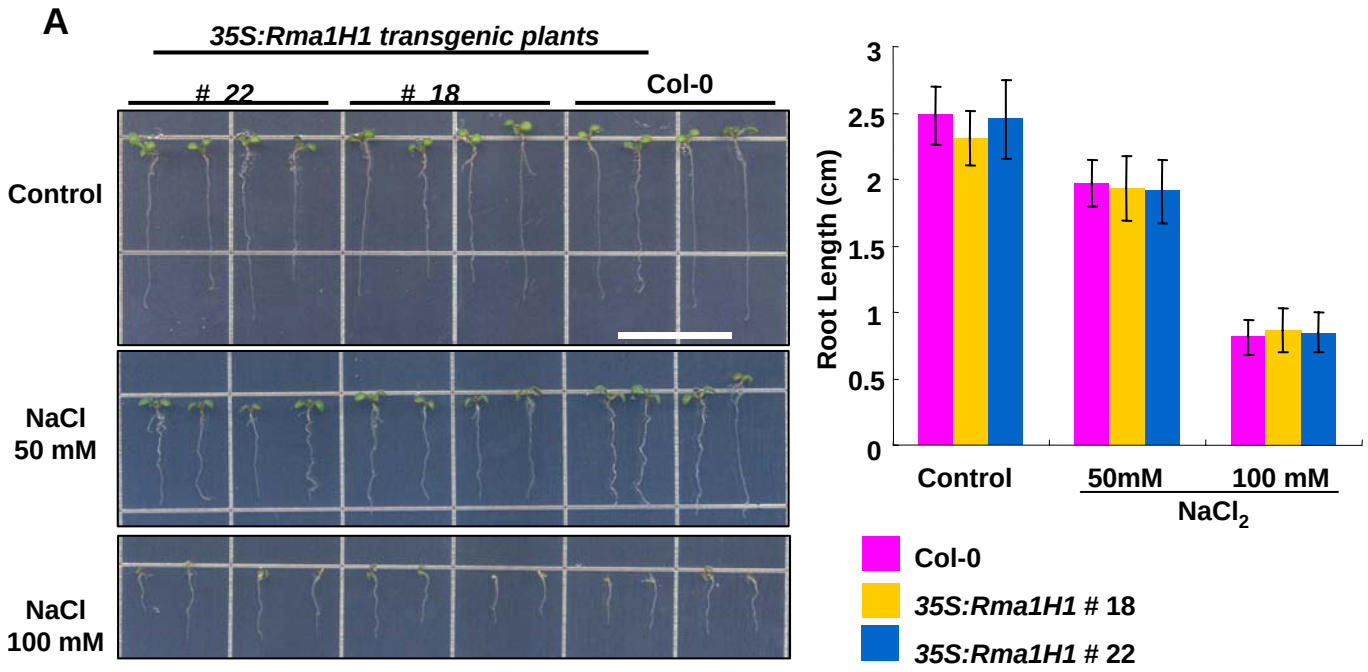

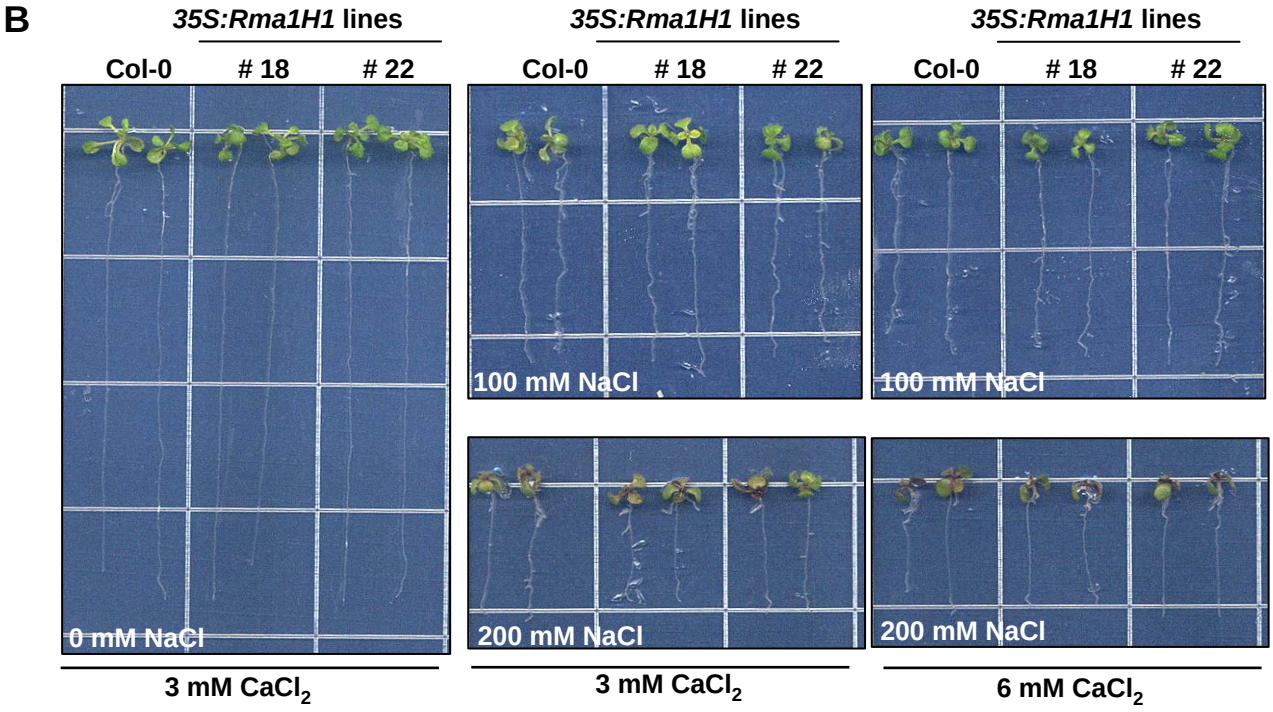

Supplemental Figure 5. Root growth patterns of wild type and *35S:Rma1H1* transgenic plants in response to salt treatment. To measure root growth, seeds of wild type and *35S:Rma1H1* T4 transgenic plants were sown on solid MS medium containing 3% (w/v) sucrose, 0.8% (w/v) phytoagar and different concentrations of $CaCl_2$ (0, 3, and 6 mM) without or with NaCl (50-100 mM). Plates containing seeds were incubated at 4^{o}C for 3 days and then transferred to a growth chamber (22^{o}C under a 16 h light/8 h dark regime) in a vertical orientation. Seedlings were further incubated in the growth chamber and then root length was monitored after 7 days. **(A)** Root growth patterns of wild type and *35S:Rma1H1* transgenic plants in response to salt treatment without $CaCl_2$. The values are means ±SD (n = 20). Bar, 1.8 cm **(B)** Root growth patterns of wild type and *35S:Rma1H1* transgenic plants in response to salt treatment in the presence of $CaCl_2$ (3 and 6 mM).

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Supplemental Table 1. Primers for PCR, Cloning, and Construction of Transgenic Plants

Primer Name	Forward Primers	Reverse Primers
Rma1H1 F1 + R1	5'-ATGGAAGTCAATGAACGATG-3'	5'-CTGCTGTTGATAACCTCGTC-3'
Rma1H1 Full F + R	5'-GAATTCATGAATCAAGATATGGCC-3'	5'-GAATTCTCAAAACAAGATTAGACATGTG-3'
Rma1H1 *Bam*H1 F + *Sac*I R	5'-GAATTCCGGAGGCTTTGCTTTTCT-3'	5'-GAGCTCTCAAAACAAGATTAGACATGTGAC-3'
Rma1H1 *Sma*I F + *Bgl*II R	5'-AAGGGCCCATGAATCAAGATATGGCC-3'	5'-TTAGATCTTCAAAACAAGATTAGACATGT-3'
Rma1H1 *Cla*I F + *Xho*I R	5'-AAATCGATATGAATCAAGATATGGCC-3'	5'-TTCTCGAGTCAAAACAAGATTAGACA-3'
Rma1H1 ΔN F	5'-GAATTCACCTTGATTCCACTCTATGG-3'	
Rma1H1 ΔC R		5'-GAATTCTCAGACTTCAGCCTTGCAAAC-3'
Rma1H1 H58A F + R	5'-TAACTTTATGCGGTGCTCTTTACTGCTGGC-3'	5'-GCCAGCAGTAAAGAGCACCGCATAAAGTTA-3'
Rma1H1 C61S F + R	5'-GCGGTCATCTTTACAGCTGGCCTTGCATT-3'	5'-AATGCAAGGCCAGCTGTAAAGATGAACGC-3'
Rma1H1 C89S F + R	5'-CGCAATGCCCTGTTAGCAAGGCTGAAGTC-3'	5'-GACTTCAGCCTTGCTAACAGGGCATTGCG-3'
Rma1H1 K115R F + R	5'-CCAAGATTCGGAGCCCTTCCTTCGGATGG-3'	5'-CCATCCGAAGGAAGGGCTCCGAATCTTGG-3'
Rma1 F1 + R1	5'-GGCACTGACATTGACATATATGAGTTGG-3'	5'-CTAAAACAAGAGAAGACACAGAACAAC-3'
Rma1 F2	5'-ATGGCCTTAGATCAATCTTTTGAAG-3'	
Rma1 RNAi-*Xba*I F + *Hind*III R	5'-TCTAGACAACGGGTTCATTTCAATAGCCC-3'	5'-AAGCTTCTAAAACAAGAGAAGACACAGAACAAC-3'
Rma1 RNAi-*Xho*I F + *Eco*RI R	5'-CTCGAGCAACGGGTTCATTTCAATAGCCC-3'	5'-GAATTCCTAAAACAAGAGAAGACACAGAACAAC-3'
Rma2 F1 + R1	5'-ATGGAGATTGAGAAGGATGAGGACG-3'	5'-TTAGAAGAGAAATAGACACATAAACAT-3'
Rma2 RNAi-*Hind*III F + *Cla*I R	5'-AAGCTTCAGTCCGGTTCAAATGTACCGAG-3'	5'-ATCGATTTAGAAGAGAAATAGACACATAAACAT-3'
Rma2 RNAi-*Eco*RI F + *Kpn*I R	5'-GAATTCCAGTCCGGTTCAAATGTACCGAG-3'	5'-GGTACCTTAGAAGAGAAATAGACACATAAACAT-3'
Rma3 F1 + R1	5'-TAGTGGATGGGAAGATATTTAGATAGAG-3'	5'-CTAGAAGAGAAGGAGGCAAAGGATGAT-3'
Rma3 F2	5'-ATGGAAGGGAACTTCTTCATCAGGT-3'	
PIP2;1 F + R	5'-GAATTCATGGCAAAGGATGTGGAA-3'	5'-GAATTCTTAGACGTTGGCAGCAC-3'
PIP2;1 *Xho*I F + *Bam*HI R	5'-AACTCGAGATGGCAAAGGATGTGGAA-3'	5'-GGATCCGACGTTGGCAGCACTTCTGAA-3'
UBA1 F + R	5'-GAATTCATGCTTCACAAGCGAGC-3'	5'-GAATTCTCACCTGAAGTAGATAG-3'
UBC8 F + R	5'-GAATTCATGGCTTCGAAACGGATC-3'	5'-GAATTCTTAGCCCATGGCATACTT-3'
UBQ10 F + R	5'- TCAATTCTCTCTACCGTGATCAAG -3'	5'- CTTCTTAAGCATAACAGAGACGAG -3'
Ubiquitin F + R	5'-ATGCAGATCTTTGTTAAGACTCTC-3'	5'-TCAACCACCACGGAGCCTGA-3'
PINII F + R	5'-GTGCCGAATTCGGCACGA-3'	5'-CTGTGTGAAGGTACGTACGGC-3'
LEAL1 F + R	5'-GCACGAGGGACTTGAGCA-3'	5'-GTGATGTTGCCAATGACTGG-3'
RCI	5'-CAACAGAAATGTTTTGGAACCC-3'	5'-CCATATGATGATCAATCACTTGGT-3'
Actin F + R (*Arabidopsis*)	5'-TACTGATTACCTCATGAAGATCCTTAC-3'	5'-AAACGATGTCTCTTTAGTTTAGAAGC-3'
T-DNA border		
4447	5'-CCGTTTCCGTTCCGTTTTC-3'	
LB29	5'-ATTTCGGAACCACCATCAAACAGGATTT-3'	
LB3	5'-ACGGTCGGTACGGGATTTTCCCAT-3'	