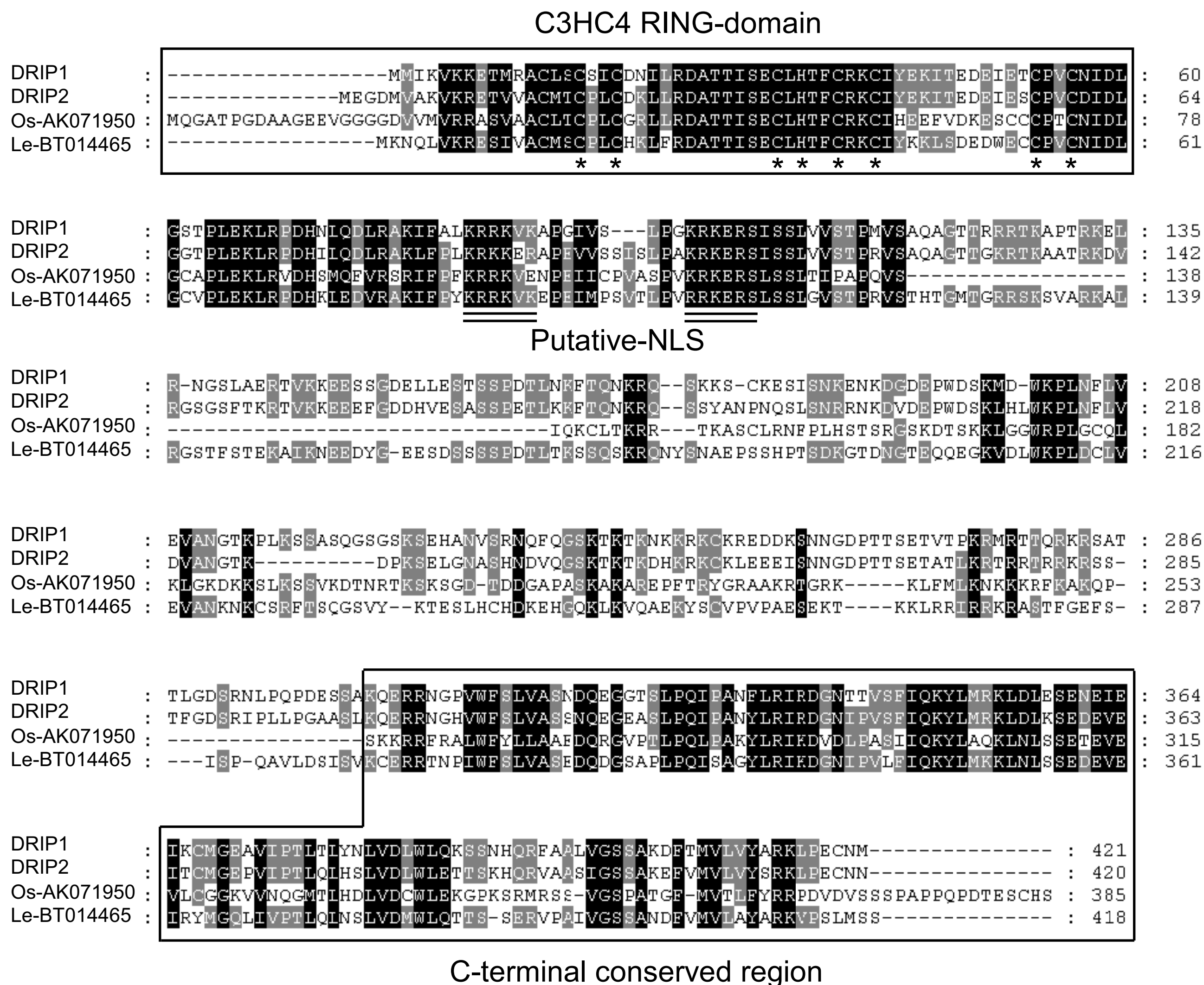
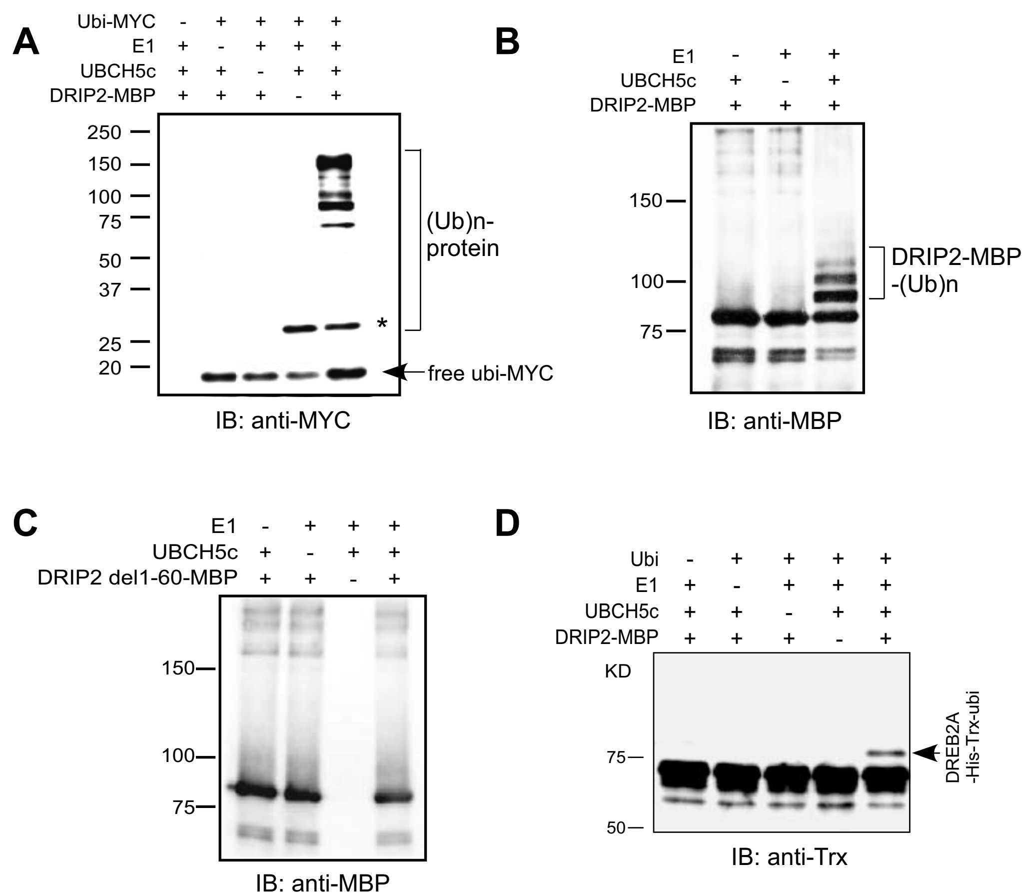
Supplemental Data. Qin et al. (2008). *Arabidopsis* DREB2A interacting proteins function as RING E3 ligases and negatively regulate plant drought stress responsive gene expression.



Supplemental Figure 1. Multiple Sequence Alignment of DRIP1, DRIP2 and Their Orthologs.

AK071950 is from *Oryza sativa*, and BT014465 is from Lycopersicon esculentum. The multi-sequence alignment was processed with ClustalW software (http://www.ebi.ac.uk/clustalw/). The putative NLS was predicted by http://wolfpsort.org/. The conserved metal-ligand residues of cysteine or histidine are marked with asterisks; the putative NLS is double underlined; and the first 60 amino acids forming the C3HC4 RING domain and C-terminal conserved domain are highlighted in boxes.



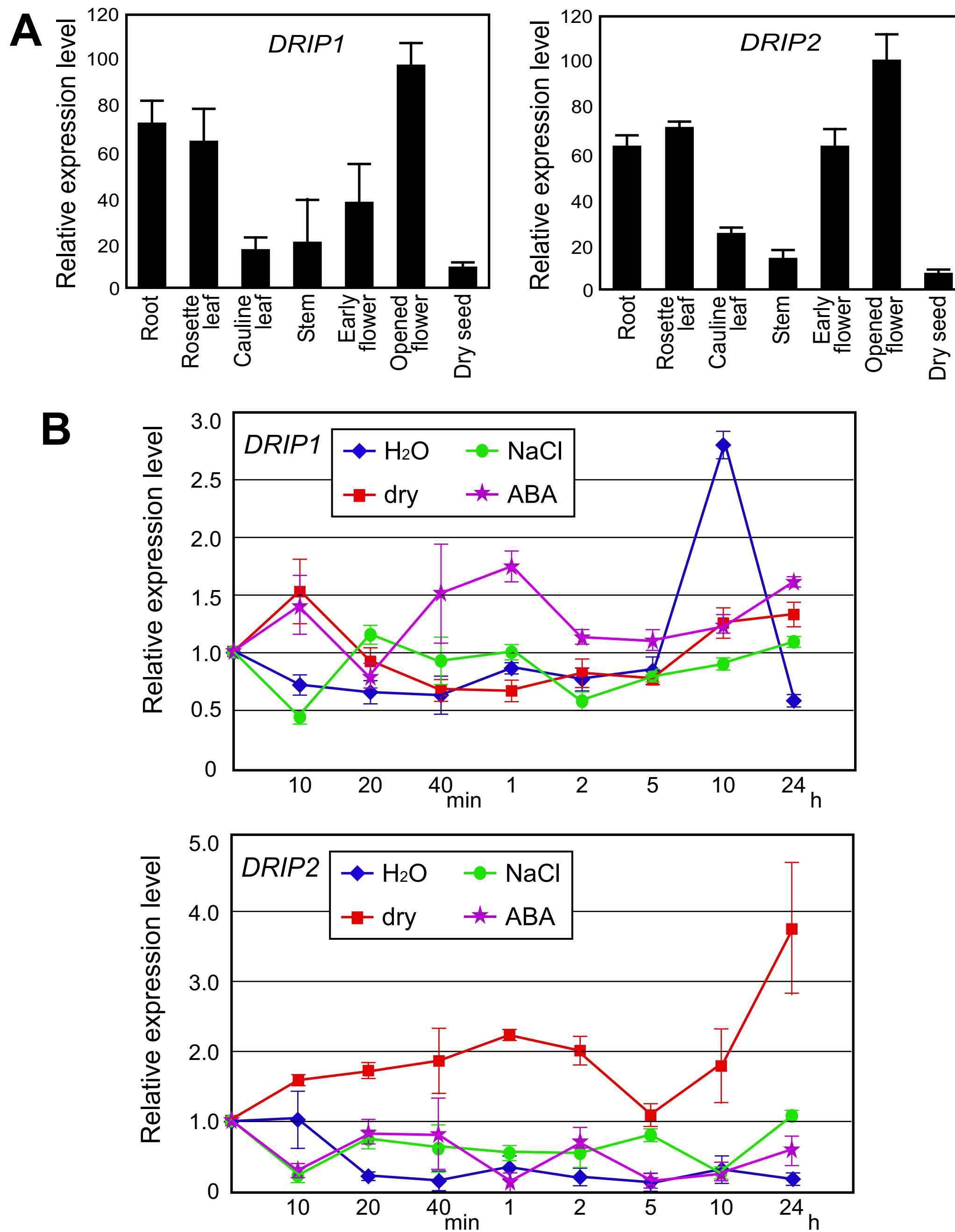
Supplemental Figure 2. DRIP2 Functions as an E3 Ubiquitin Ligase and Mediated DREB2A Protein Ubiquitination.

(A) In the presence of UBCH5c as E2, DRIP2-MBP fusion protein displayed ubiquitin E3 ligase activity. The ubiquitin attached protein bands were detected by anti-myc immunoblot (10% SDS-PAGE). The asterisk marker indicated the E2-dependent ubiquitination bands.

(B) Auto-ubiquitination of DRIP2-MBP was detected at the presence of UBCH5c. MBP antibody was used to detect DRIP2-MBP fusion protein, and the shifted band in the last line indicated ubiquitin molecues attached to DRIP2-MBP protein (6% SDS-PAGE).

(C) The wildtype and RING domain deleted DRIP2-GST fusion protein (DRIP2 del1-60-MBP) E3 ubiquitin ligase activity was tested at the presence of UBCH5c (E2), E1 and ubiquitin. MBP antibody was used to detect DRIP2-MBP fusion protein (6% SDS-PAGE).

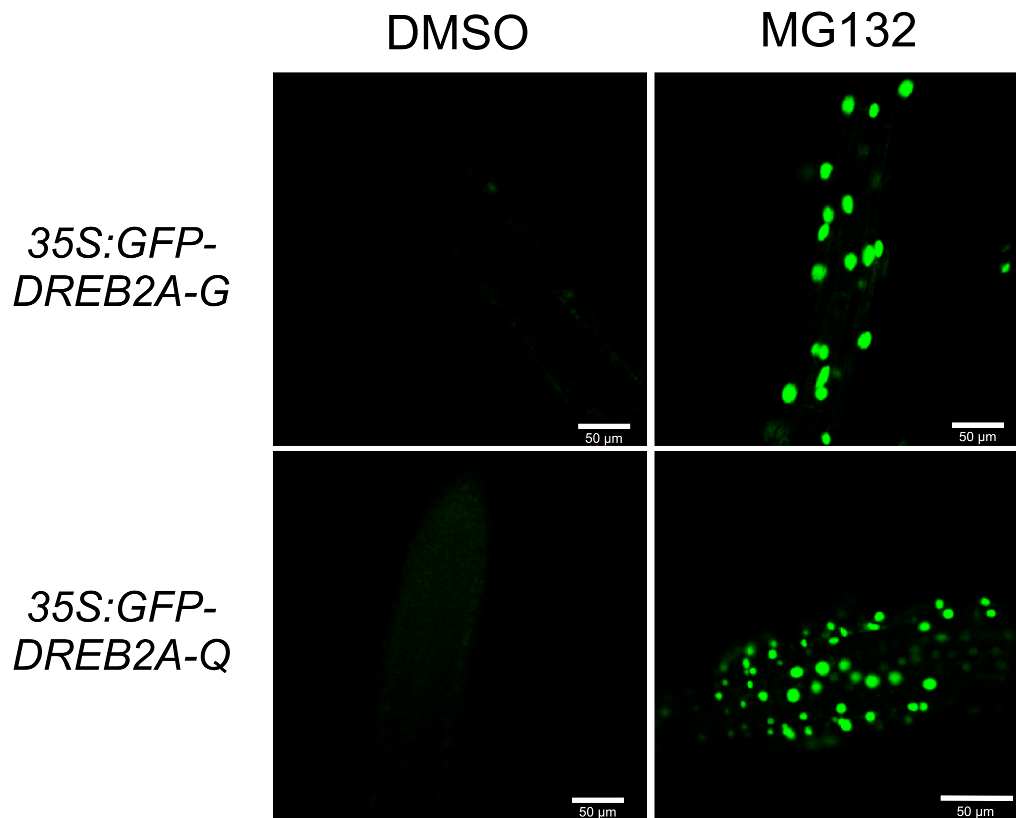
(D) DRIP2 mediates ubiquitination of DREB2A protein. The full-length DREB2A protein was fused with a His and Trx tag (DREB2A-His-Trx) and was used as the substrate for the in vitro assay. Anti-Trx was used in the immunoblot for the detection of Trx-tagged substrate protein (6% SDS-PAGE).



Supplemental Figure 3. *DRIP1* and *DRIP2* Gene Expression Profiling under Various Stresses in Different Tissues.

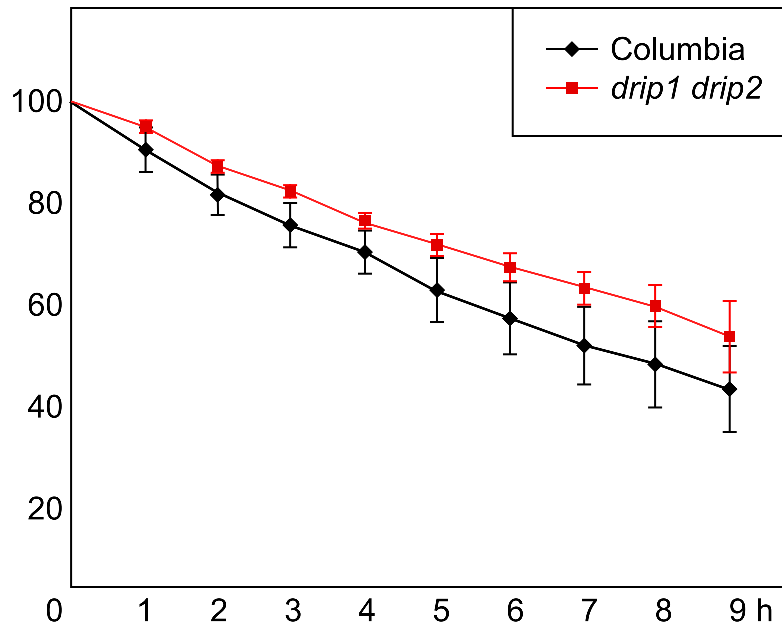
(A) qRT-PCR PCR analysis of *DRIP1* and *DRIP2* gene expressions under normal conditions in various tissues. Roots and rosette leaves were obtained from 3-week-old plants; cauline leaves and stems were harvested from inflorescences of 9-week-old plants; early flowers were in flowering stages 7-10; opened flowers in flowering stages 11-13. Dry seeds were collected from a well-matured seed stock which exhibited a good germination rate. All materials were obtained from the Columbia wild type background.

(B) qRT-PCR analyses of *DRIP1* and *DRIP2* gene expressions under various stress conditions. 3-week-old Columbia plants growing on agar plates were subjected to stress treatments (250 mM NaCl; dehydration; 100 μM ABA) and a H2O treatment was used as a control. Gene expression under 0 h was designated as 1.0, respectively.



Supplemental Figure 4. Proteasome inhibitor MG132 inhibits GFP-DREB2A fusion protein degradation when it is overexpressed by 35S promoter in *Arabidopsis.*

Two independent transgenic lines, *35S:GFP-DREB2A-G* and *Q*, were treated by DMSO (mock treated) or proteasome inhibitor MG132 for 15 hrs and observed under confocal microscope. Bars indicate 50 μm.



Supplemental Figure 5. Relative Water Content of Wild-type Columbia and *drip1 drip2* Double Mutant under Dehydration Conditions.

The aerial parts of 4-week-old soil-grown plants were detached and dehydrated on empty dishes in 65%±5% relative humidity. The standardized water content was calculated as described in Fujita et al. (2005). Each data point represents the mean of duplicate measurements (n=8). Error bars represent standard deviation.

Qin et al. Supplemental Table 1. The Primer List Used in qRT-PCR

no.	pimer	5' - 3'	Note
1	DREB2A RealT F	GACCTAAATGGCGACGATGT	
2	DREB2A RealT R	TCGAGCTGAAACGGAGGTAT	
3	18S rRNA-F	AAACGGCTACCACATCCAAG	
4	18S rRNA-R	CCTCCAATGGATCCTCGTTA	
5	DRIP1-RealT F1	GAATTTGCCACAACCAGATGAG	for realtime PCR in Columbia
6	DRIP1-RealT R1	GCCACAAGTGAGAACCAAACG	for realtime PCR in Columbia
7	DRIP1-RealT F2	CCGTGACGCCACCACTATC	for realtime PCR in *drip1-1*
8	DRIP1-RealT R2	CATCCTCCGTGATCTTCTCATAGA	for realtime PCR in *drip1-1*
9	DRIP2-RealT F	TTTTCTTGTGGATGTGGCAAAC	
10	DRIP2-RealT R	TTTACTCCCCTGAACATCATTGTGG	
11	Promoter-DRIP1-F	GGGGGATCCAGGAGATGACCTAGAGGAACC	
12	Promoter-DRIP1-R	GGGGGATCCCTTTCTCTTTATCTGTTGGGC	
13	DREB2A 165aa-MYC-STOP-NOTI-R	TTTGCGGCCGCTCACAGATCTTCTTCAGAAATAAGTTTTTGTT CCTCTGTTTTCACATGAACAC	
14	SALK_145041-LP	TTGTTGGTGGCGAACTTATTC	
15	SALK_145041-RP	TGAAACCAGTAAAGCCGTGTC	
16	WiscDsLox437G06-LP	TCTCTCCTTCCTCTTTCCTGG	
17	WiscDsLox437G06-RP	GCTTAAGCTGCTCTATCTGCG	
18	T-DAN left border	GCGTGGACCGCTTGCTGCAACT	
19	At rd29A-F	TGGACACGAATTCTCCATCA	
20	At rd29A-R	TTCCAGCTCAGCTCCTGATT	
21	At rd29B-F	GGAGAGAGCAGAGAGGCTCA	
22	At rd29B-R	CCGTTGACCACCGAGATAGT	
23	rd17-F	ACGTCCACGCCGTTGGT	
24	rd17-R	CTCCGGATGTTCCACTGGAA	
25	At1g52690-F	GCAATCAAGAACAAGGCACA	
26	At1g52690-R	TCAGTGCGAAGCCCTAAAGT	
27	LEA14-F	GATTTCTTCTGATCGACAAAACCTA	
28	LEA14-R	AGCAAACCCAACTTATTACATTACG	

29	AT3g17520-F	GGTTTGGTTATGGTATCTTTGGTACTTA	
30	AT3g17520-R	CTTTGCCCAATCAGTCCATGAT	
31	AtGolS1 At2g47180-F	CTTGGAAAAGTCAAGGTGGTTCA	
32	AtGolS1 At2g47180-R	TCGCTTCTTTCCCTGTGTATCTC	
33	AtGolS2 At1g56600-F	GACGAGTCTCTTGATTACAAGAATGTT	
34	AtGolS2 At1g56600-R	AAACTGCTGAAGTGTCTGTTGC	
35	At2g21490-F	GGCAAACACAAGGACGAACAA	
36	At2g21490-R	TCGTGAGGTTGGTCGGTAGTG	

Supplemental Table 2. Positive Clones from Library Screening

Clone NO.	-His-Ade	β-gal	20 mM 3-AT	AGI code	MIPS annotation	Function of its homolog in other organism
17	+	+++	+++	At1g06770	C3HC4 Ring domain/zinc finger like protein	E3 ubiquitin ligase activity
36	+	+	+/-	At3g51260	multicatalytic endopeptidase complex alpha chain	
77	+	+	+	At1g53280	cytoplasmic and nuclear protein degradation	
23	+	+	+/-	At1g70300	high affinity potassium transporter AtHAK6	
6	+	+	-	At2g35500	unknown protein, predicted to be targeted to chloroplasts	shikimate kinase precursor, tomato
21	+	+	-	At2g35500	unknown protein, predicted to be targeted to chloroplasts	shikimate kinase precursor, tomato
2	+	+	+	At4g27750	unknown protein	impaired sucrose induction 1-like protein [Solanum tuberosum].
14	+	+	+	At4g27750	unknown protein	impaired sucrose induction 1-like protein [Solanum tuberosum].
3	+	+/-	++	At3g46780	unknown protein, predicted to be targeted to chloroplasts	NADH2 dehydrogenase (ubiquinone) I chain nueM Aquifex aeolicus
4	+	+	+	At3g46780	unknown protein, predicted to be targeted to chloroplasts	NADH2 dehydrogenase (ubiquinone) I chain nueM Aquifex aeolicus
5	+	+	+	At3g46780	unknown protein, predicted to be targeted to chloroplasts	NADH2 dehydrogenase (ubiquinone) I chain nueM Aquifex aeolicus
9	+	+	-	At3g46780	unknown protein, predicted to be targeted to chloroplasts	NADH2 dehydrogenase (ubiquinone) I chain nueM Aquifex aeolicus
7	+	+	+	At4g36980	unknown protein, predicted to be targeted to mitochondria	likely to be a transmembrane protein
10	+	+	+	At4g36980	unknown protein, predicted to be targeted to mitochondria	likely to be a transmembrane protein
26	+	+	+	At4g36980	unknown protein, predicted to be targeted to mitochondria	likely to be a transmembrane protein
48	+	+	+	At4g36980	unknown protein, predicted to be targeted to mitochondria	likely to be a transmembrane protein
12	+	+	++	At4g28910	unknown protein	putative RNA-binding protein
22	+	+	+	At1g21560	hypothetical protein	putative RNA helicase
24	+	++	+	At3g04480	unknown protein	endoribonuclease L-PSP family protein-like, rice
25	+	-	-	At1g20960	putative ATP-dependent RNA helicase	
49	+	-	-	At1g20960	putative ATP-dependent RNA helicase	
19	+	+	-	At3g25920	50S ribosomal protein L15, chloroplast precursor	
13	+	+	-	At3g25920	50S ribosomal protein L15, chloroplast precursor	
30	+	+/-	-	At3g25920	50S ribosomal protein L15, chloroplast precursor	
27	+	+/-	-	At1g31817	30S ribosomal like protein S11	
33	+	+/-	-	At3g49080	30S ribosomal protein S9 -like	
68	+	-	-	At3g49080	30S ribosomal protein S9 -like	
37	+	+/-	-	At1g20100	unknown protein	putative RNA-binding protein
41	+	+	-	At3g04260	hypothetical protein	putative DNA binding domain containing protein, rice
1	+	++	++	At1g54440	hypothetical protein,Targeted to secretory pathway	ribonuclease III Brucella melitensis (strain 16M)
16	+	+		At1g54440	hypothetical protein,Targeted to secretory pathway	ribonuclease III [imported] Brucella melitensis (strain 16M)
35	+	-	-	At5g13430	ubiquinol--cytochrome-c reductase - like protein	
74	+	-	-	At5g13430	ubiquinol--cytochrome-c reductase - like protein	
52	+	-	-	At5g13430	ubiquinol--cytochrome-c reductase - like protein	
40	+	+	-	At1g21130	O-methyltransferase like protein	
38	+	+/-	-	At1g31180	3-isopropylmalate dehydrogenase like protein	
75	+	-	*	At1g68560	alpha-xylosidase precursor	
28	+	++	++	At3g15580	unknown protein	putative microtubule-associated protein
76	+	-	*	At4g15880	unknown proein	putative microtubule-associated protein
66	+	-	*	At5g18440	unknown protein	nuclear FMRP interacting protein 1 NUFIP1, Homo sapiens, EMBL:AF159548
15	+	+	*	At2g30350	unknown protein	
32	+	+	*	At4g26630	unknown protein	

+' indicates cell growth or positive signals; '-' indicates no cell growth or negative signals; '*' indicates no data.

Description of pGreen Vectors

pGKX:

To remove EcoRV site between the El2 and the CaMV 35S promoter sequences in pBE2113 (Mitsuhara et al., 1996), two DNA fragments containing a portion of EI2-35SΩ cDNA were generated by PCR with the following two pairs of primers: forward primer A, 5’-GACCATGATTACGCCAAGCTTGAAC-3’; reverse primer A, 5’-GTGGAGACAGCACATCAATCCAC-3’; forward primer B, 5’-GATGTGCTGTCTCCACTGACGTAAG-3’; reverse primer B, 5’-GATATCTCGAGCTCGCGGCCGCCCCGGGGAT-3’. The resulting purified fragments A and B were mixed in a tube for PCR, denatured at 94 °C for 10 min, annealed, and polymerized at 72 °C for 3 min. Then, a DNA fragment amplified in the tube with forward primer A and reverse primer B was directly inserted into pGreen-NOS cassette (Hellens et al., 2000) at XbaI site, in which XbaI cleavage was blunted with T4 DNA polymerase. The resultant plasmid, pGreen-NOS-EI2-35S, was digested with HindIII and SphI to give a 1128-bp fragment. The HindIII-SphI cleavage site was blunted with T4 DNA polymerase. The 1128-bp fragment was then inserted into pGreen II 0029 at a ApaI-SacI site, in which ApaI-SacI cleavage was also blunted with T4 DNA polymerase, producing pGreen II 0029-EI2-35SΩ (named pGKX).

pGKX-NsGFP:

To gain the sGFP cDNA (Chiu et al., 1996), PCR was performed on pGFP3BX (Fujita et al., 2004) using the primer pair 5’-GGGACTAGTATGGTGAGCAAGGGCGAG-3’ and 5’-GGGTCTAGATGCTTGTACAGCTCGTCCAT-3’. The sGFP cDNA fragment was inserted into pGKX at XbaI site to generate pGK-El2-35SΩ-NsGFP (named pGKX-NsGFP).

pGKX-CsGFP:

pGFP3BX (Fujita et al., 2004) was digested with BamHI and PstI to give a 743-bp fragment. The BamHI-PstI cleavage site was blunted with T4 DNA polymerase. The 743-bp fragment was cloned into pGK-EI2-35SΩ at a XhoI site, in which XhoI cleavage was also blunted with T4 DNA polymerase, to generate pGK-EI2-35SΩ-CsGFP (named pGKX-CsGFP).

pGK-GUS:

To gain the GUS-CaMV terminator fragment, PCR was performed on pGreen-35S-GUS cassette (Hellens et al., 2000) using the primer pair 5'-TATGTTACGTCCTGTAGAAACC-3' and 5'-GATCTGGATTTTAGTACTGG-3', producing a 2120-bp fragment. The 2120-bp fragment was directly inserted into pGreenII 0029 (Hellens et al., 2000) at an ApaI site, in which ApaI cleavage was blunted with T4 DNA polymerase, producing pGreen II 0029-GUS (named pGK-GUS).

Supplemental References

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