

Supplementary Material

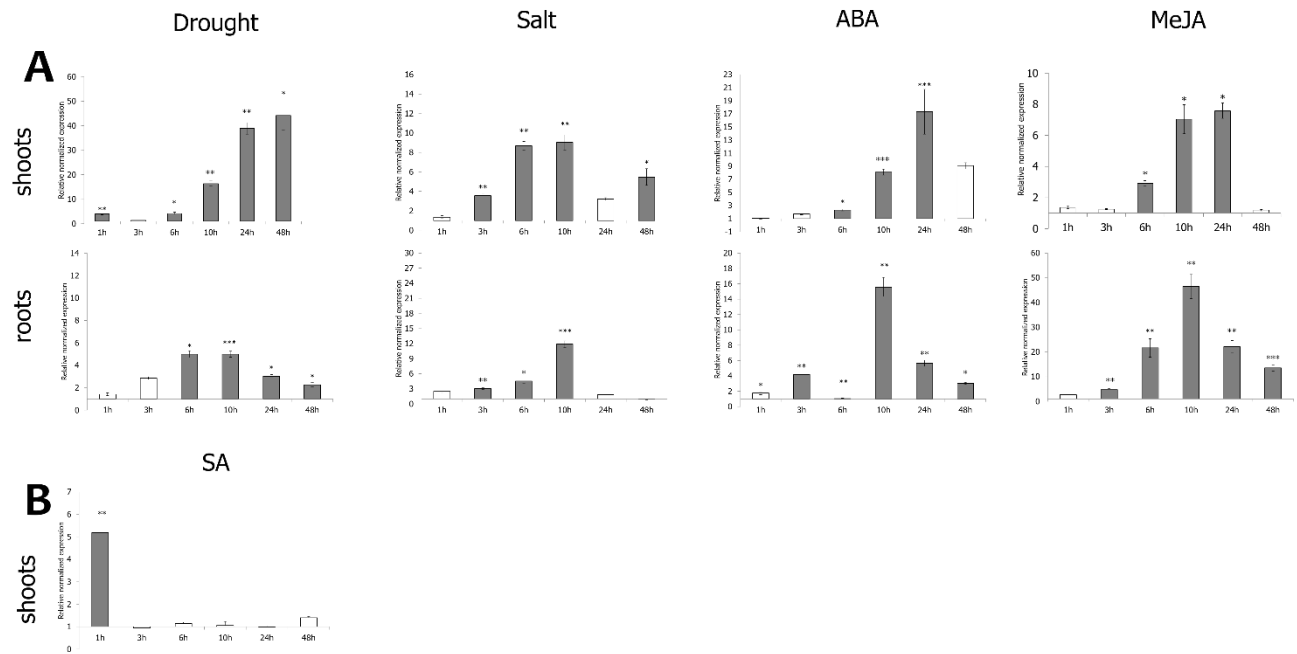
EUL gene expression in rice: stress regulation, subcellular localization and tissue specificity.

Jeroen Lambin, Sinem Demirel Asci, Malgorzata Dubiel, Mariya Tsaneva, Isabel Verbeke, Pieter Wytynck, Jeroen De Zaeytijd, Guy Smagghe, Kondeti Subramanyam and Els J. M. Van Damme*

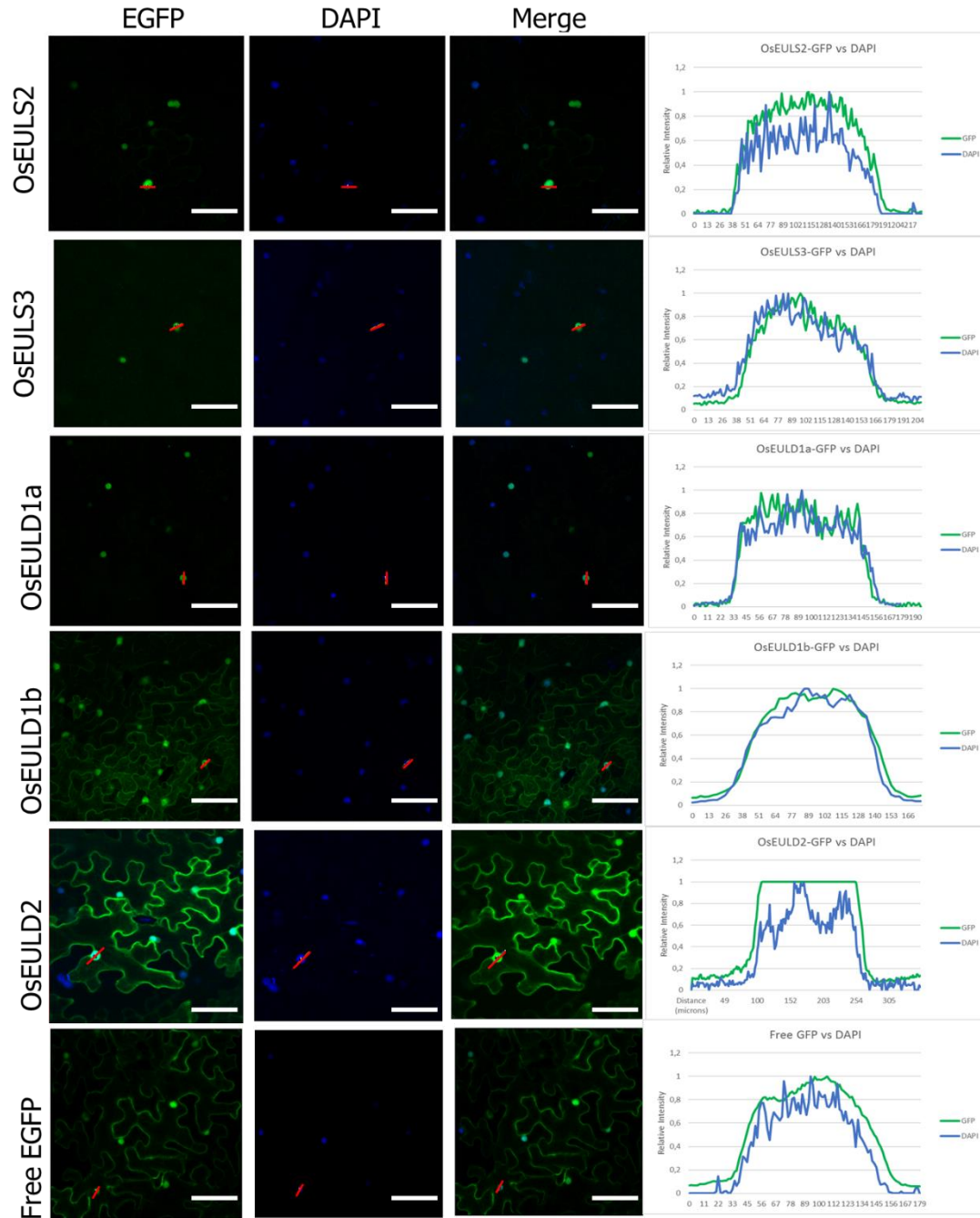
*** Correspondence:** Prof dr Els JM Van Damme: elsjm.vandamme@ugent.be

1 Supplementary Figures and Tables

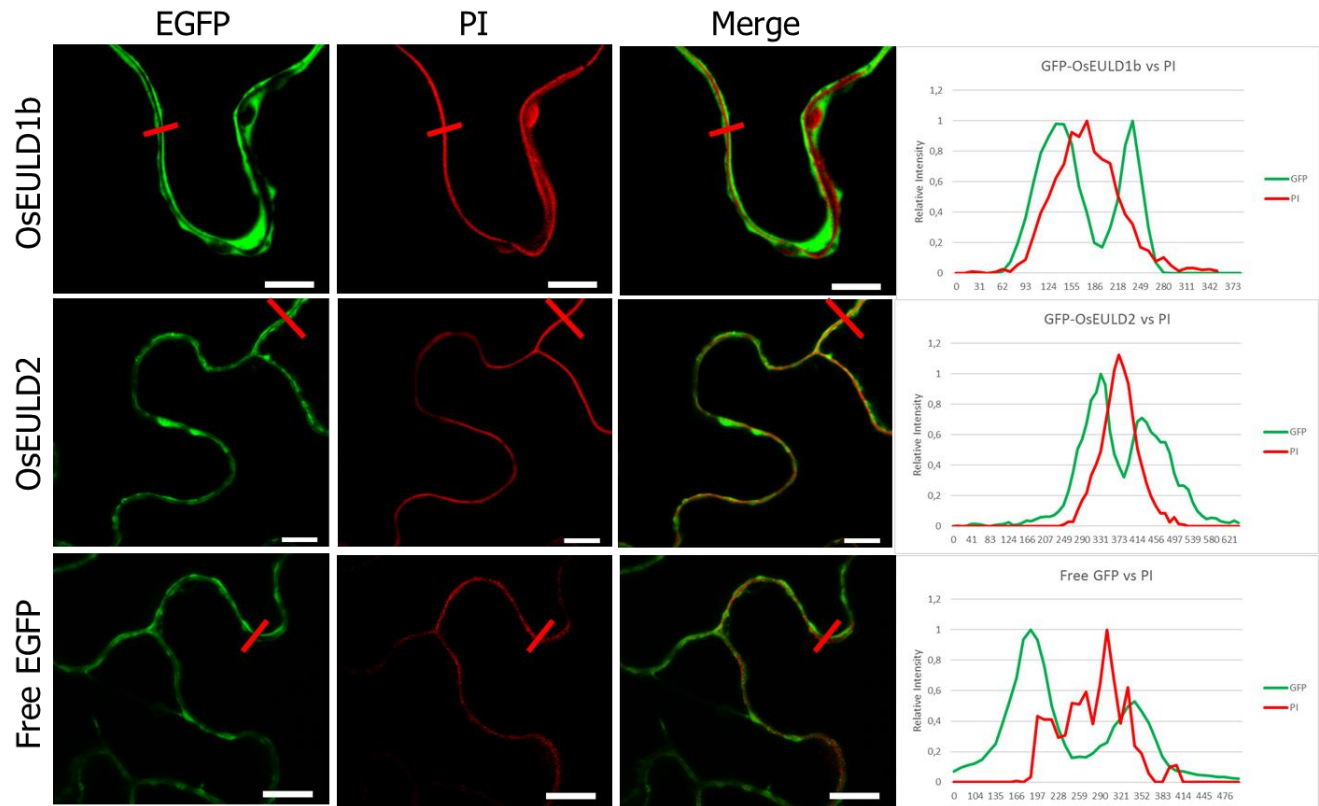
1.1 Supplementary Figures



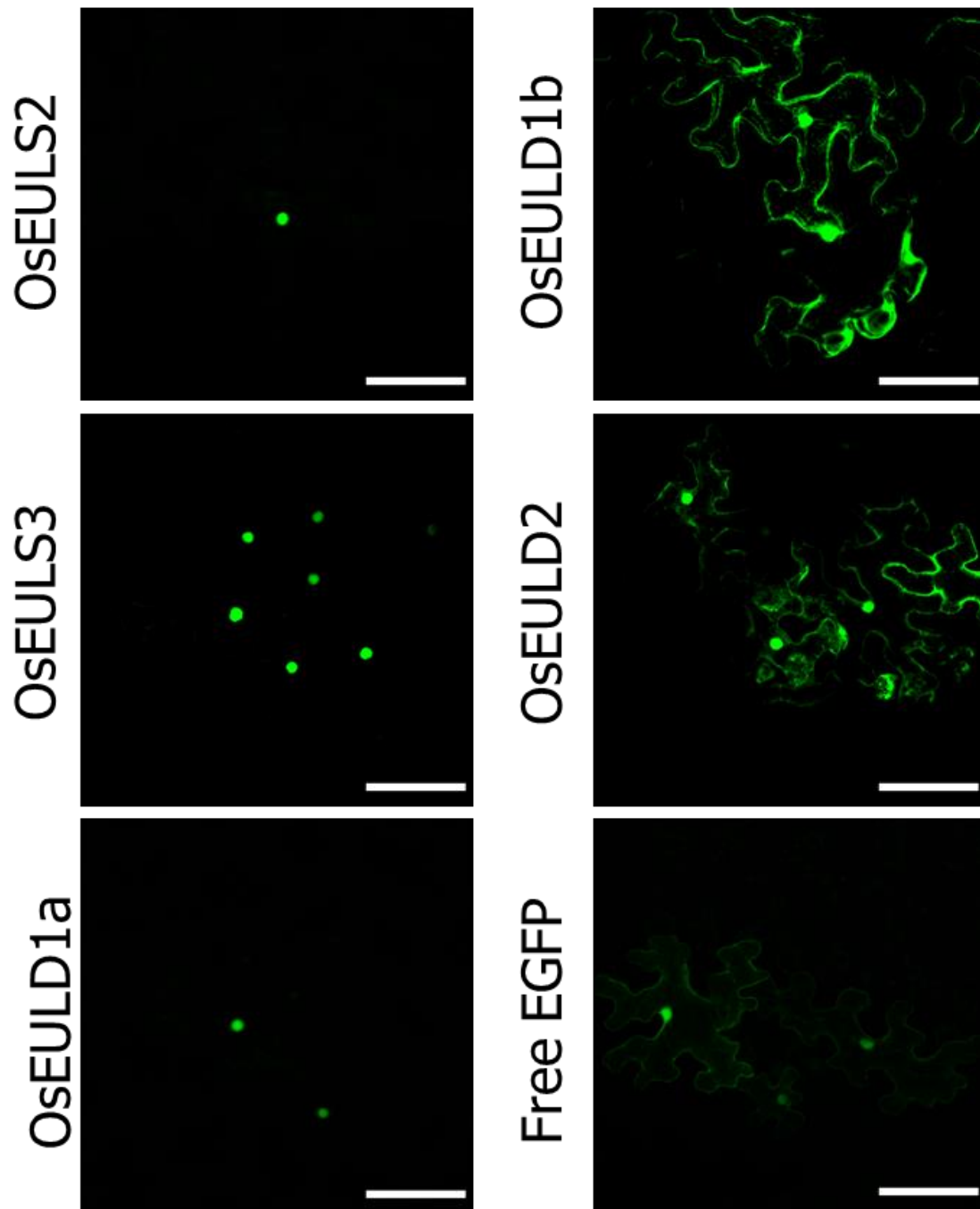
Supplementary Figure 1. Controls for the stress conditions imposed by different hormonal and abiotic stress treatments. Orysat is used as positive control for drought, salt, ABA and MeJA treatment (A). WRKY45 is used as a positive control for SA treatment (B). Normalized expression levels, relative to the control (non-treated plants, set to 1), are shown for different time points. The mean values of RT-qPCR for three independent biological replicates were normalized to three reference genes and error bars indicate standard errors. Dark-gray bars represent statistically significant up-regulation of at least two-fold compared to non-treated plants and light-gray bars represent statistically significant down-regulation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).



Supplementary Figure 2. Subcellular localization of OsEUL-EGFP and free EGFP in *N. benthamiana* lower epidermal cells. Nuclei were stained with DAPI. Co-localization between DAPI and EGFP signals was assessed in overlay pictures (Merge). Graphs show the plot profiles of the eGFP and DAPI fluorescent signals across the nuclei. The red line shows the part of the picture being plotted. Microscopy settings were identical for all pictures. Scale bars represent 80 μm.



Supplementary Figure 3. Zoom in of subcellular localization of OsEULD1b, OsEULD2 and free EGFP in *N. benthamiana* lower epidermal cells. Cell walls were stained with PI. Co-localization of PI and EGFP staining was assessed in overlay pictures (Merge). Graphs show the plot profiles for eGFP and PI fluorescent signals at the border region between neighboring cells. The red line shows the part of the picture being plotted. Microscopy settings were identical for all pictures. Scale bars represent 10 μm .



Supplementary Figure 4. Subcellular localization of OsEUL-EGFP and free EGFP in *N. benthamiana* lower epidermal cells after ABA treatment (50 μM) for 1h. Microscopy settings were identical for all pictures. Scale bars represent 80 μm .

1.2 Supplementary Tables

Supplementary Table 1. Overview of gene specific primers used for RT-qPCR

Gene	LOC number	Sequence (5' to 3')	Description	Primer efficiency
Exp	LOC_Os03g27010	TGTGAGCAGCTTCTC GTTTG	forward qPCR primer to amplify a reference gene EXP	2.122
		TGTTGTTGCCTGTGA GATCG	reverse qPCR primer to amplify a reference gene EXP	
EIF5C	LOC_Os011g21990	CACGTTACGGTGAC ACCTTTT	forward qPCR primer to amplify a reference gene EIF5C	2.024
		GACGCTCTCCTTCTT CCTCAG	reverse qPCR primer to amplify a reference gene EIF5C	
Exp Narsai	LOC_Os07g02340	AGGAACATGGAGAA GAACAAGG	forward qPCR primer to amplify a reference gene EXPNarsai	1.981
		CAGAGGTGGTGCAG ATGAAA	reverse qPCR primer to amplify a reference gene EXPNarsai	
OsEULS2	LOC_Os07g48500	CATCAAGCACTCCCT CGGCCAGTC	Forward primer for OsEULS2	1.934
		CTGAACCCGTTCCCC ACGTCGCGG	Reverse primer for OsEULS2	
OsEULS3	LOC_Os01g01450	CGCTCGACTGGCCCT AGCTAAGTG	Forward primer for OsEULS3(in the 3' UTR)	1.962
		GACGTGACATGTTC ATATTCGTACCG	Reverse primer for OsEULS3(in the 3' UTR)	
OsEULD1a	LOC_Os07g48490	AACAGTGGGTGATG TAAGTGCAGG	Forward primer for OsEULD1A(in the 3' UTR)	2.002
		GGGTGCGAGACAAAT GAGCCATTC	Reverse primer for OsEULD1A (in the 3' UTR)	
OsEULD1b	LOC_Os03g21040	CCGTGATCTGTGGA GTTGG	Forward primer for OsEULD1B (in the 3' UTR)	2.023
		GCAGGACTCGAGAA AACGAC	Reverse primer for OsEULD1B (in the 3' UTR)	
OsEULD2	LOC_Os07g48460	TCGAGAGACCGTCA ACAAAA	Forward primer for OsEULD2 (in the 3' UTR)	2.023
		GGACACGCAACAGT AACACG	Reverse primer for OsEULD2 (in the 3' UTR)	
Orysata	LOC_Os01g24710	CGAAATAATGTTCC ATGGTGT	Forward primer for positive control Oryzata (in the 3' UTR)	1.999
		TGTACTACGGATCG GTGCAA	Reverse primer for positive control Oryzata (in the 3' UTR)	
WRKY45	LOC_OS05G25770	GATGGAGGACATGG AGAAGG	Forward primer for positive SA control WRKY45 (in the 3' UTR)	1.932
		GAGAAGGTGTGGAG AATCTGG	Reverse primer for positive SA control WRKY45 (in the 3' UTR)	

Supplementary Table 2. Overview of primers used in molecular cloning of the GUS reporter lines

Gene	LOC number	Sequence (5' to 3')	Description
OsEULS2	LOC_Os07g48500	CGGGGTACCATGCCCACAAA CGAGCCATAG	forward primer+KpnI site to amplify pOSEULS2
		GCCGCTCGAGGCTTCTTGATTT ATGACTCGCTAG	reverse primer+XhoI site to amplify pOSEULS2
OsEULS3	LOC_Os01g01450	CGGGGATCCGGCCGCTGATGTA TTGACTTGT	forward primer+BamHI site to amplify pOSEULS3
		GCCCTCGAGGATGGATGGATTT GGGGGAATG	reverse primer+XhoI site to amplify pOSEULS3
OsEULD1a	LOC_Os07g48490	CGGGGTACCTCCACAGGACAA CCTACACATAG	forward primer+KpnI site to amplify pOSEULD1a
		GCCGCTCGAGCCCGAACCCAA ACATCTTC	reverse primer+XhoI site to amplify pOSEULD1a
OsEULD1b	LOC_Os03g21040	CGGGGTACCGCCTTGCCTTTGA CGTG TAG	forward primer+KpnI site to amplify pOSEULD1b
		GCCGCTCGAGGTGTTGTGTGAG TGTGATGGATC	reverse primer+XhoI site to amplify pOSEULD1b
OsEULD2	LOC_Os07g48460	CGGGGTACCGACATAGCTGCTG CTCGATTG	forward primer+KpnI site to amplify pOSEULD2
		GCCGCTCGAGTCTGGTTGTCTC GCTGGTATTC	reverse primer+XhoI site to amplify pOSEULD2

Supplementary Table 3. Overview of primers used in molecular cloning of the GFP vectors

Gene	Loc number	Sequence (5' to 3')	Description
OsEULS2	LOC_Os03g21040	AAAAAGCAGGCTTCACCATG GACTTTTACGGGCGGCGCGA	forward primer for OsEULS3 CDS with non-complete attb1 site
		AGAAAGCTGGGTGTCAGTA GTAGGGCTGGATCTTCCAGC G	reverse primer for OsEULS2 CDS with non-complete attb2 site (+ stop codon for C-tag)
		AGAAAGCTGGGTGGTAGTA GGGCTGGATCTTCCAGCG	reverse primer for OsEULS2 CDS with non-complete attb2 site (- stop codon for N-tag)
OsEULS3	LOC_Os07g48460	AAAAAGCAGGCTTCTCTTCT CCCTTTTGCTATCTCTA	forward primer for OsEULS3 CDS with non-complete attb1 site
		AGAAAGCTGGGTGCCAGGG AACAATCTTCCAGC	reverse primer for OsEULS3 CDS with non-complete attb2 site (+ stop codon for C-tag)
		AGAAAGCTGGGTGCCAGGG AACAATCTTCCAGC	reverse primer for OsEULS3 CDS with non-complete attb2 site (- stop codon for N-tag)
OsEULD1a	LOC_Os07g48490	AAAAAGCAGGCTTCACCATG TTTGGGTTCGGGCACCACCA	forward primer for OsEULD1a CDS with non-complete attb1 site (+ stop codon for C-tag)
		AGAAAGCTGGGTGCTACCA GGGGAGGATCTTCCAGCGCT G	reverse primer for OsEULD1a CDS with non-complete attb2 site (+ stop codon for C-tag)
		A GAA AGC TGG GTG CCA GGG GAG GAT CTT CCA GCG CTG	reverse primer for OsEULD1a CDS with non-complete attb2 site (- stop codon for N-tag)
OsEULD1b	LOC_Os03g21040	AAAAAGCAGGCTTCACCATG TTCGGCTTCGGGCACCACGG CC	Forward primer for OsEULD1b CDS with incomplete attB1 site

		AGAAAGCTGGGTGCCAGGG GACGATCTTCCAGCGCTG	Reverse primer for OsEULD1b CDS with incomplete attB2 site (+ stop codon for C-tag)
		A GAA AGC TGG GTG TTA CCA GGG GAC GAT CTT CCA GCG CTG	Reverse primer for OsEULD1b CDS with incomplete attB2 site (- stop codon for N-tag)
OsEULD2	LOC_Os07g48460	AAAAAGCAGGCTTCACCATG TTCAGCCACCATGGGCACGG C	Forward primer for OsEULD2 CDS with incomplete attB1 site (+ stop codon for C-tag)
		AGAAAGCTGGGTGCCAGGG GAGGATCTTCCAGCGCTG	Reverse primer for OsEULD2 CDS with incomplete attB2 site (+ stop codon for C-tag)
		A GAA AGC TGG GTG TTA CCA GGG GAG GAT CTT CCA GCG CTG	Reverse primer for OsEULD2 CDS with incomplete attB2 site (- stop codon for N-tag)
AttB sites		GGGGACAAGTTTGTACAAA AAAGCAGGCT	rest of AttB1-site
		GGGGACCACTTTGTACAAGA AAGCTGGGT	rest of AttB2-site