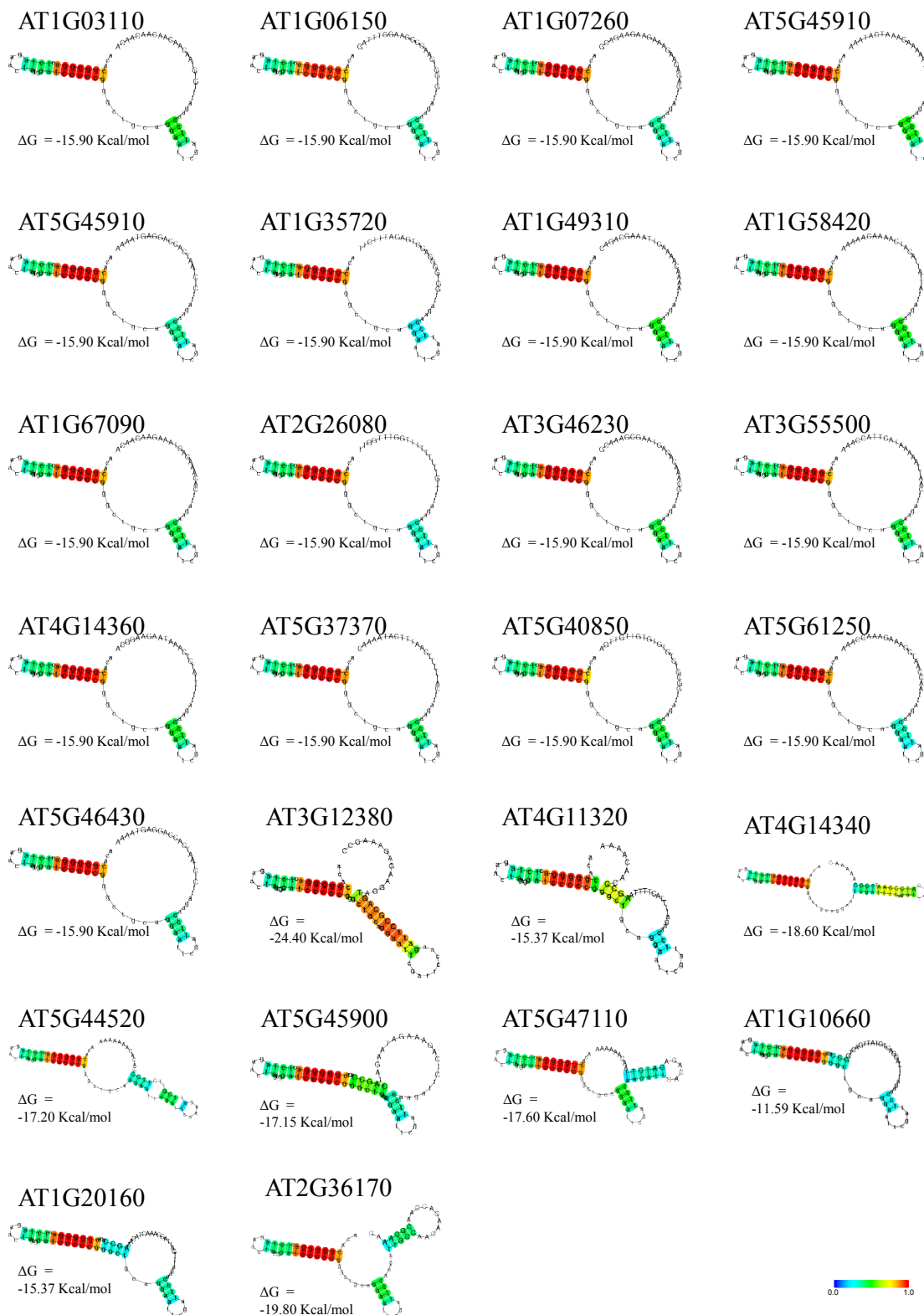


## Supplemental Figures

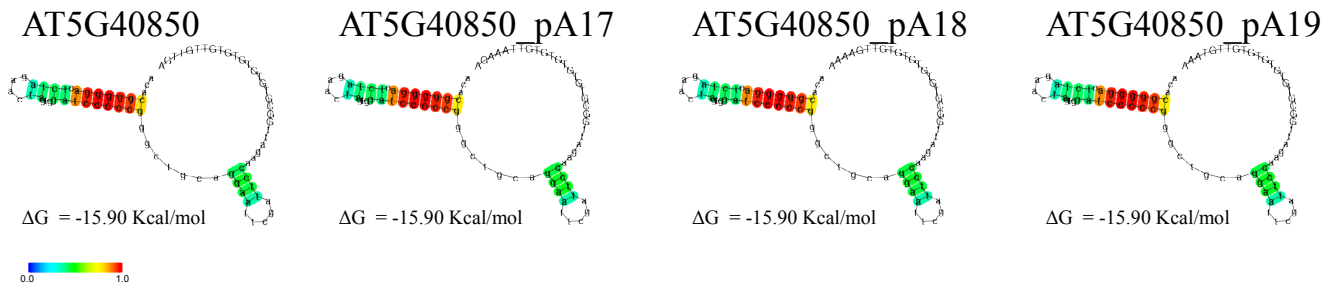
### Supplemental Figure S1. The secondary structure analysis of various 5'-UTR constructs used in this study.

The entire 5'-UTR consisting of the 165 nucleotide 5'-UTR from the expression vector and the 21 nucleotide region of the 5'-UTR from various Arabidopsis genes was analyzed for the possible secondary structure using centroidfold (<http://www.ncrna.org/centroidfold/>). The 21 nucleotide 5'-UTRs from various Arabidopsis genes are in the capital letters. The probability of the secondary structure formation was color-coded. Heat color gradation from blue to red represents the base-pairing probability from 0 to 1. The base-pairing probability is the probability that a pair of bases forms a base-pair via hydrogen bonds in the secondary structure.



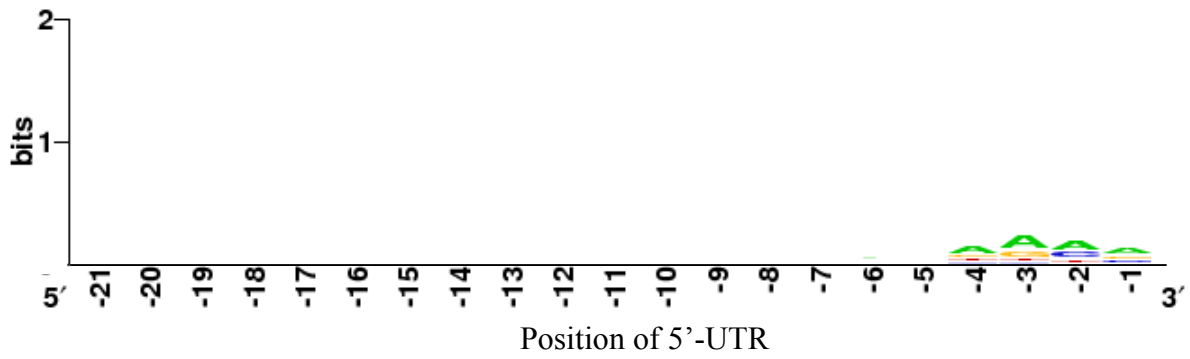
**Supplemental Figure S2. The secondary structure analysis of triple A substitution mutants of the AT5G40850 5'-UTR constructs used in this study.**

The entire 5'-UTR consisting of the 165 nucleotide 5'-UTR from the expression vector and the 21 nucleotide region of the wild-type or triple A substitution mutants of the AT5G40850 5'-UTR was analyzed for the possible secondary structure using centroidfold (<http://www.ncrna.org/centroidfold/>). The probability of the hairpin formation was color-coded. Heat color gradation from blue to red represents the base-pairing probability from 0 to 1. The wild-type or triple A substitution mutants of the 21 nucleotide AT5G40850 5'-UTR are in the capital letters.



**Supplemental Figure S3. Sequence analysis of 5'-UTRs in the entire Arabidopsis genes.**

The 5'-UTRs of the entire Arabidopsis genes were analyzed using weblogo (<http://weblogo.berkeley.edu/>) to identify any preferred nucleotides in the 5'-UTR region from positions -1 to -21.



Supplemental Tables

Supplemental Table S1. The nucleotide sequences of primers for vector construction

group	decription	sequence 5' → 3'	Lower case letter	Upper case
P1	RbcS1A_5'UTR_10nt	AAGAAGAACatgagtaaaggagaagaact	overlapping sequences for smGFP	5'UTR
	RbcS1A_5'UTR_21nt	CACAAAGAGTAAAGAAGAACAatgagtaaaggagaagaact	overlapping sequences for smGFP	5'UTR
P2	smGFP_3'	tattgttatattcatcca	overlapping sequences for smGFP	-
P3	RbcS1A_5'UTR_46nt	AAAGCACTAGACCAAACTCAGTCACacaaagagtaaaggagaagaact	overlapping sequences for RbcS1A_5'UTR_21nt	5'UTR
	RbcS1A_5'UTR_72nt	GTTGTTATCATTATATATAGATGACCAaagcactagaccaaacct	overlapping sequences for RbcS1A_5'UTR_46nt	5'UTR
	RbcS1A_5'UTR_94nt	CACCTCAAAGCTTGTCTCATTgtgtttatcattatataatagatg	overlapping sequences for RbcS1A_5'UTR_72nt	5'UTR
	RbcS1A_5'UTR_121nt	AATCTCATGTATCTAGACCTCCGATcactcaaagctgttct	overlapping sequences for RbcS1A_5'UTR_94nt	5'UTR
	RbcS1A_5'UTR_147nt	CAACGGGTAGATCTTAGTCCACTTgaatctcatgtatctagac	overlapping sequences for RbcS1A_5'UTR_121nt	5'UTR
	RbcS1A_5'UTR_175nt	AACCTCACATATCCACTCAAATCcaacgggtgtagatccta	overlapping sequences for RbcS1A_5'UTR_175nt	5'UTR
P4	AT1G35720_5'-UTR	AACACTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G45900_5'-UTR	CTCAGA AAGATAAGATCGCCatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G61250_5'-UTR	AACCAATCGAAAGAACCAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G46430_5'-UTR	CTCTAATCACCAGGAGTAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G47110_5'-UTR	GAGAGAGATCTTAACAAAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G03110_5'-UTR	TGTGTAACAACAACAACAACAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT3G12380_5'-UTR	CCGAGTAGGAAGAGAAAGCCatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G45910_5'-UTR	AAAAAAAAGAAATCATAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G58420_5'-UTR	ATTATTACATCAAAAACAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT5G44520_5'-UTR	CGTTCTCCACACAAAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G07260_5'-UTR	GAGAGAAGAAAGAAAGAGCAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT3G55500_5'-UTR	CAATTAATAACTTACCAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT3G46230_5'-UTR	GCAACACAGTAAGCCAAACAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT2G36170_5'-UTR	GCGAAGAAAGCAAGCAAGCAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G10660_5'-UTR	TTAGGACTGTATTGACTGGCAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT4G14340_5'-UTR	ATCATCGGAATTCGAAAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G49310_5'-UTR	AAAAAAAAGTTAAAGCAGACatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT4G14360_5'-UTR	TTTATCTCAAATAAGAGGCAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G28520_5'-UTR	GGTGGGGAGGTGAGATTCTTatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	AT1G20160_5'-UTR	TGATTAGAAACTACAAAGCAtgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
AT5G37370_5'-UTR	CATTTTTCAATTTTCATAAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR	
AT4G11320_5'-UTR	TTACTTTTAAGCCCAACAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR	
AT5G40850_5'-UTR	GGCGTGTGTGTGTGTGTGAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR	
AT1G06150_5'-UTR	TGGTGAAGGGGAAGGTTTGAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR	
AT2G26080_5'-UTR	TTGTTTTTTTTGGTTTGGTTatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR	
P5	Good UTR Triple Adenine_1	AAAACATAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_2	AAAAATAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_3	AACAAAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_4	AACACAAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_5	AACACTAAAAATAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_6	AACACTAAAAAAGAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_7	AACACTAAAAAAGAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_8	AACACTAAAAAGTAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Adenine_9	AACACTAAAAAGTAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
P6	Good UTR Triple Thymine_1	TTTACTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_2	ATTTCTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_3	AATTTTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_4	AACTTTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_5	AACATTTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_6	AACACTTTAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_7	AACACTTTTAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_8	AACACTATTTGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_9	AACACTAATTTTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_10	AACACTAAATTTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_11	AACACTAAATTTGAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_12	AACACTAAAAGTTTAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_13	AACACTAAAAGTTTAAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_14	AACACTAAAAGTATTTGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_15	AACACTAAAAGTAGTTTTAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_16	AACACTAAAAGTAGATTTAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_17	AACACTAAAAGTAGAATTTAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_18	AACACTAAAAGTAGAAGTTTAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Thymine_19	AACACTAAAAGTAGAAGATTTatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
P7	Good UTR Triple Guanine_1	GGGACTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_2	AGGGCTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_3	AGGGGTAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_4	AACGGGAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_5	AACAGGGAAAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_6	AACACGGGAAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_7	AACACTGGGAGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_8	AACACTAGGGGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_9	AACACTAAGGGTAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_10	AACACTAAAGGGGAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_11	AACACTAAAAGGGGAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_12	AACACTAAAAGTGGGAGAAGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_13	AACACTAAAAGTAGGGGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_14	AACACTAAAAGTAGAGGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_15	AACACTAAAAGTAGAAGGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_16	AACACTAAAAGTAGAAGGGAAAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Guanine_17	AACACTAAAAGTAGAAGAGGGAatgagtaaaggagaaga	overlapping sequences for smGFP	5'UTR

P8	Good UTR Triple Cytosine_1	CCCCTAAAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_2	ACCCCTAAAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_3	AACCCCTAAAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_4	AACCCCAAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_5	AACACCCAAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_6	AACACCCCAAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_7	AACACTCCCAGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_8	AACACTACCCGTAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
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	Good UTR Triple Cytosine_10	AACACTAAACCAGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_11	AACACTAAAACCCGAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_12	AACACTAAAAGCCCCAAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_13	AACACTAAAAGTCCCAGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_14	AACACTAAAAGTACCCGAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_15	AACACTAAAAGTAGCCCAAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_16	AACACTAAAAGTAGACCCAAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_17	AACACTAAAAGTAGAACCCAAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_18	AACACTAAAAGTAGAAGCCCAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Good UTR Triple Cytosine_19	AACACTAAAAGTAGAAGACCAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
P9	Poor UTR Triple Adenine_1	AAAGTGTGTGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_2	GAAATGTGTGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_3	GGAAGTGTGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_4	GGCAAATGTGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_5	GGCGAAAGTGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_6	GGCGTAAATGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_7	GGCGTAAAAGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_8	GGCGTGAATGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_9	GGCGTGAAGTGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_10	GGCGTGTGTAATGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_11	GGCGTGTGTAAGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_12	GGCGTGTGTGTAATGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_13	GGCGTGTGTGTAATGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_14	GGCGTGTGTGTAAAGTGTGTTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_15	GGCGTGTGTGTGAAATGTAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_16	GGCGTGTGTGTGTAAATGTAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_17	GGCGTGTGTGTGTAAAGTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_18	GGCGTGTGTGTGTGAAAGTGAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
	Poor UTR Triple Adenine_19	GGCGTGTGTGTGTGTAATGTAAtgagtaaaggagaagaa	overlapping sequences for smGFP	5'UTR
P10	UTR-good:TRP1_5'	ATTATTACATCAAAACAAAAtgtctgttattattcacagg	overlapping sequences for TRP I	5'UTR
	UTR-poor:TRP1_5'	GGCGTGTGTGTGTGTTGTAAtgtctgttattattcacagg	overlapping sequences for TRP I	5'UTR
P11	TRP1_3'	TCAAGCGTAATCTGGAACATCGTATGGGTAttcttagcttttgacgaaatt	overlapping sequences for TRP I	HA tag
P12	UTR-good:NPT II_5'	ATTATTACATCAAAACAAAAtgattgaaacaagatgattgca	overlapping sequences for NPT II	5'UTR
	UTR-poor:NPT II_5'	GGCGTGTGTGTGTGTTGTAAtgattgaaacaagatgattgca	overlapping sequences for NPT II	5'UTR
P13	NPT II-3'	tcagaagaactctgaagaag	overlapping sequences for NPT II	-

Supplemental Table S2. The nucleotide sequences of primers for qRT

<b>Target</b>	<b>name of primer</b>	<b>sequence</b>
smGFP	GFP_q5	AAGAGCGCCATGCCTGAGGG
	GFP_q3	GCTCGATCCTGTTGACGAGGG
ACT2 (At3g18780)	ACT2_q5	TATGAATTACCCGATGGGCAAG
	ACT2_q3	TGGAACAAGACTTCTGGGCAT
$\beta$ -glucuronidase	GUS_q5	CAAAGCGGCGATTTGGAA
	GUS_q3	GCCAGGCCAGAAGTTCTTTT
hygromycin phosphotransferase	HYG_q5	GATGTTGGCGACCTCGTATT
	HYG_q3	ATTTTCGGCTCCAACAATGTC