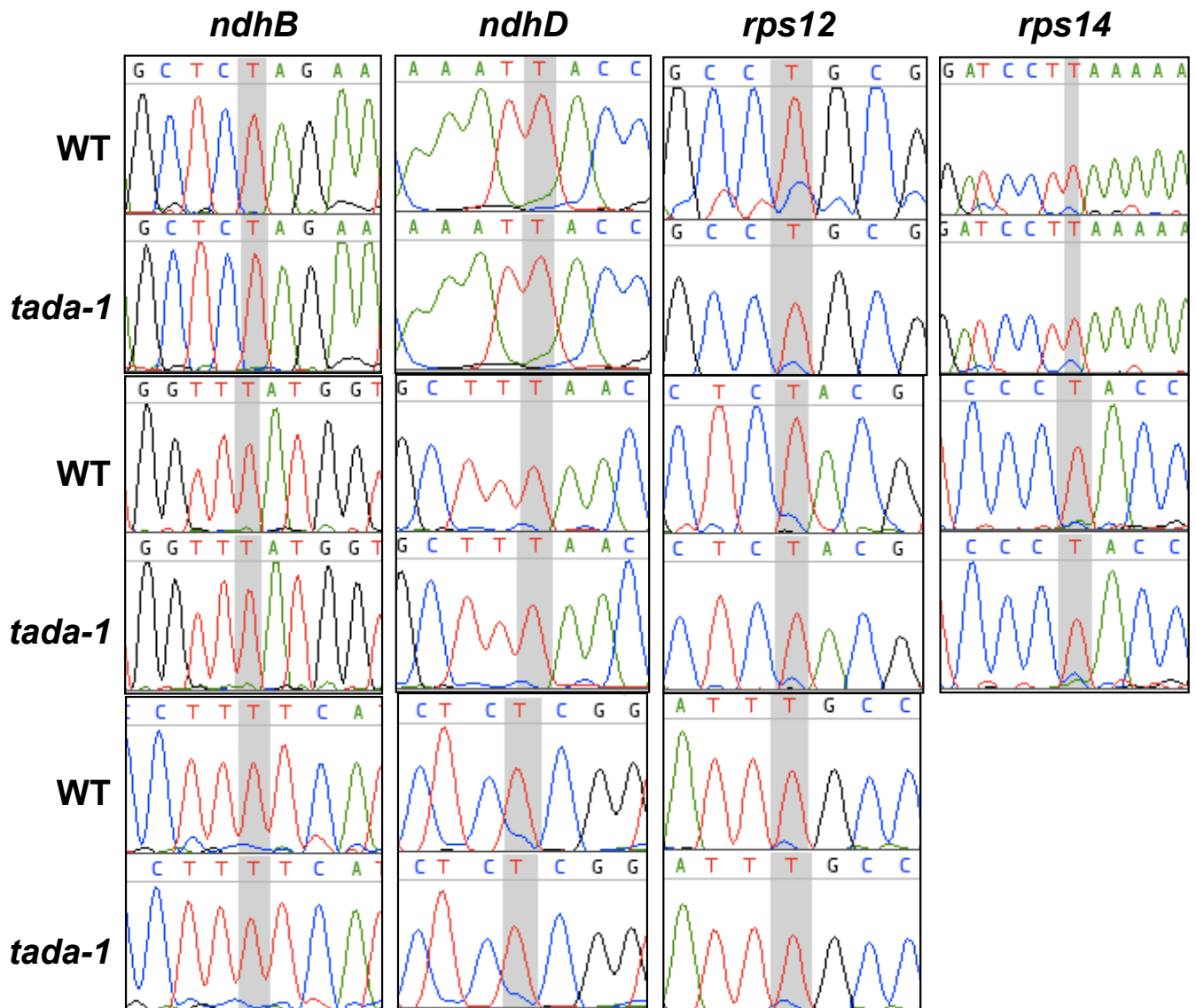
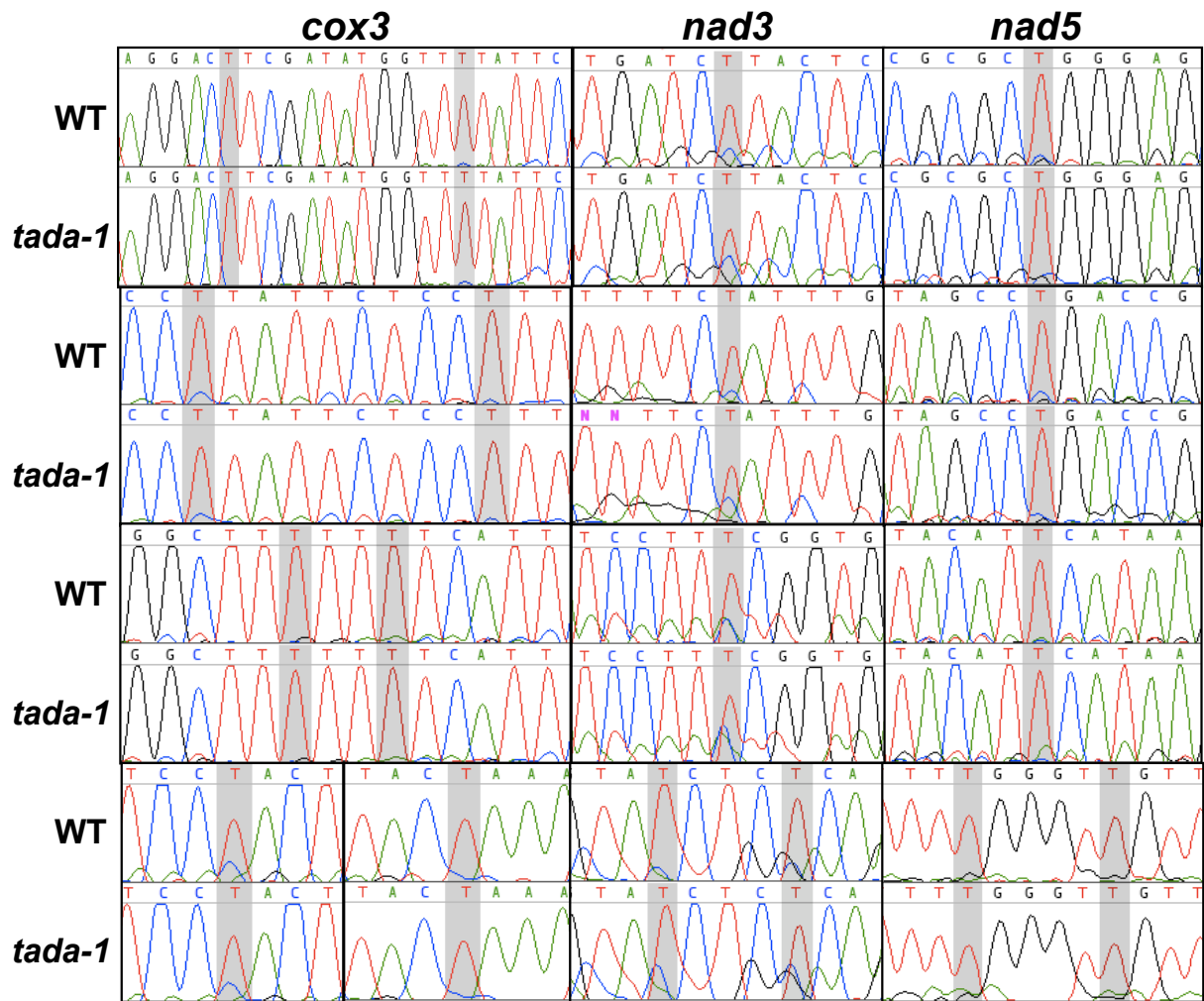


**Supplemental figure 4.** RNAi plants deficient in *TADA* expression are also affected in cp-tRNA<sup>Arg</sup> editing. (A) Phenotypes of slow growth of several individual plants of the RNAi line that expresses a *TADA*-specific RNA stem-loop. Plants that were further analyzed are numbered. (B) RT-PCR analysis of *TADA* gene mRNA accumulation in the selected plants. C is negative control. (C) Analysis of tRNA anticodon editing in selected plants shows that editing deficiency correlates with *TADA* silencing.

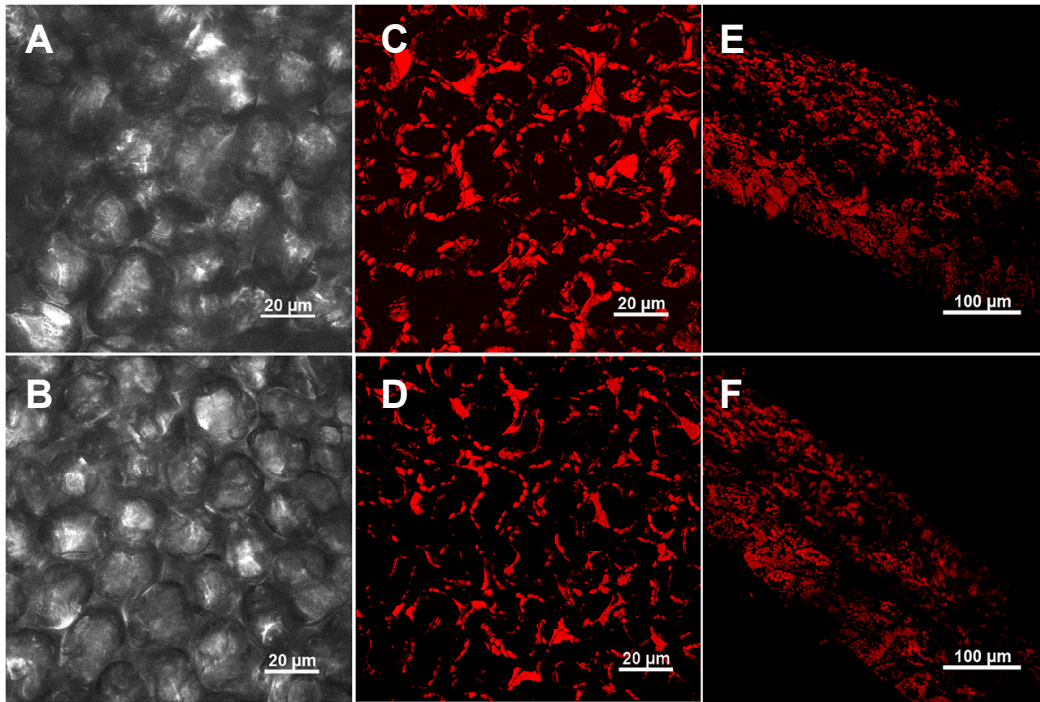


**Supplemental figure 5.** TADA is not involved in chloroplast C-to-U editing. Regions of several chloroplast gene transcripts comprising known editing sites were amplified by RT-PCR and sequenced. No difference could be found between *tada-1* and wild-type Col-0 plants.



**Supplemental figure 6.** TADA is not involved in mitochondrial C-to-U editing. Regions of several mitochondrial gene transcripts comprising editing sites were amplified by RT-PCR and sequenced. All 82 sites analyzed on 6 different genes are edited in *tada-1*. Only a few examples are shown.





**Supplemental figure 7.** *tada-1* plants have normal leaf structure and equivalent number of chloroplasts per cell as wild type plants.

(A and B) Confocal microscope image of mesophyll cells of wild type (A) and *tada-1* (B) leaves (adaxial side) observed under visible light.

(C and D) Confocal images of the fluorescence of chlorophyll in leaves of wild type (C) and *tada-1* (D) plants suggest that there are no significant differences in the number of chloroplasts per cell in *tada-1*.

(E and F) The same as in C and D, in cross sections of wild type (E) and *tada-1* (F) leaves.

**Supplemental Table 1**Chlorophyll content of *tada-1* plants

	<i>μg/mg FW</i>			<i>mol/mol</i>
	chl a	chl b	chl a+b	a/b
WT	1.29 + 0.12	0.41 + 0.05	1.70 + 0.16	3.25 + 0.30
<i>tada-1</i>	0.54 + 0.09	0.17 + 0.04	0.71 + 0.12	3.27 + 0.27



## Supplemental Table 2

Similarities between the N-terminal and C-terminal domains  
of plant TADA proteins

Full length protein

IdentityScores(%)

	Arabidopsis	Phiscomytrella	Rice	Soybean
Arabidopsis	100.0	10.7	18.4	33.1
Phiscomytrella	14.5	100.0	7.9	11.9
Rice	32.5	11.7	100.0	18.2
Soybean	47.5	16.1	30.3	100.0

Similarity Scores (%)

C-term domain

IdentityScores(%)

	Arabidopsis	Phiscomytrella	Rice	Soybean
Arabidopsis	100.0	51.2	64.9	75.4
Phiscomytrella	61.0	100.0	48.1	50.2
Rice	78.2	62.0	100.0	66.3
Soybean	81.4	60.5	80.7	100.0

Similarity Scores (%)

N-term domain

IdentityScores(%)

	Arabidopsis	Phiscomytrella	Rice	Soybean
Arabidopsis	100.0	2.7	13.7	24.1
Phiscomytrella	6.1	100.0	2.0	3.5
Rice	28.8	4.7	100.0	12.5
Soybean	39.8	6.8	25.9	100.0

Similarity Scores (%)

Supplemental Table 3

Accumulation of chloroplast transcripts in *tada-1* and *tada-1* complemented with  $\Delta$ N-TADA compared to Col-0

AGI number	gene name	<i>tada-1</i>		$\Delta$ N-TADA		AGI number	gene name	<i>tada-1</i>		$\Delta$ N-TADA	
		log <sub>2</sub> (mut/WT)	std deviation	log <sub>2</sub> (mut/WT)	std deviation			log <sub>2</sub> (mut/WT)	std deviation	log <sub>2</sub> (mut/WT)	std deviation
AtCg00020	<i>psbA</i>	0,8054	0,0491	-0,1595	0,0442	AtCg00650	<i>rpS18</i>	1,9344	0,2780	-0,2243	0,0764
AtCg00040	<i>matK</i>	2,3189	0,1101	0,5093	0,0480	AtCg00660	<i>rpL20</i>	2,2311	0,0343	-0,1046	0,0530
AtCg00065	<i>rpS12A</i>	1,3696	0,3918	-0,0344	0,0403	AtCg00670	<i>clpP1</i>	1,3675	0,2835	-0,4446	0,0365
AtCg00070	<i>psbK</i>	0,9578	0,1955	0,2535	0,1647	AtCg00680	<i>psbB</i>	0,8433	0,1387	0,1190	0,0466
AtCg00080	<i>psbI</i>	1,2850	0,0902	0,2482	0,0267	AtCg00690	<i>psbT</i>	1,0385	0,1910	0,3544	0,0214
AtCg00120	<i>atpA</i>	-0,1907	0,2887	-0,0109	0,0442	AtCg00700	<i>psbN</i>	0,9276	0,1431	-0,3166	0,0345
AtCg00130	<i>atpF</i>	0,0510	0,3267	0,1497	0,0347	AtCg00710	<i>psbH</i>	1,2012	0,1858	0,1782	0,0542
AtCg00140	<i>atpH</i>	1,0965	0,1554	0,1458	0,0485	AtCg00720	<i>petB</i>	1,3280	0,1569	-0,0650	0,0573
AtCg00150	<i>atpI</i>	1,2991	0,0879	0,3405	0,0765	AtCg00730	<i>petD</i>	1,4878	0,0986	-0,1895	0,0671
AtCg00160	<i>rpS2</i>	2,1155	0,0213	0,1483	0,0891	AtCg00740	<i>rpoA</i>	1,8270	0,0805	0,2034	0,0103
AtCg00170	<i>rpoC2</i>	1,7943	0,0804	-0,0408	0,0462	AtCg00750	<i>rpS11</i>	1,6219	0,1498	0,1208	0,0447
AtCg00180	<i>rpoC1</i>	2,3223	0,3357	0,0172	0,0819	AtCg00760	<i>rpL36</i>	1,6840	0,0868	0,1828	0,0776
AtCg00190	<i>rpoB</i>	2,0063	0,1816	0,0638	0,0297	AtCg00770	<i>rpS8</i>	1,8282	0,1574	0,1747	0,0522
AtCg00210	<i>petN</i>	0,5397	0,1392	0,2790	0,0429	AtCg00780	<i>rpL14</i>	2,0004	0,1015	0,1008	0,0844
AtCg00220	<i>psbM</i>	1,2521	0,1890	0,1488	0,0643	AtCg00790	<i>rpL16</i>	2,2788	0,2590	0,2985	0,0170
AtCg00270	<i>psbD</i>	0,1718	0,3309	-0,3185	0,0345	AtCg00800	<i>rpS3</i>	2,2956	0,0504	0,1111	0,0677
AtCg00280	<i>psbC</i>	0,3961	0,2312	-0,0289	0,0080	AtCg00810	<i>rpL22</i>	2,4849	0,1794	0,2469	0,2123
AtCg00300	<i>psbZ</i>	0,5647	0,1839	-0,3080	0,0267	AtCg00820	<i>rpS19</i>	2,1272	0,1741	0,1292	0,2021
AtCg00330	<i>rpS14</i>	1,2863	0,1106	-0,1024	0,0121	AtCg00830	<i>rpL2</i>	1,9490	0,3350	0,1127	0,0429
AtCg00340	<i>psaB</i>	0,5365	0,1639	0,0621	0,0494	AtCg00840	<i>rpL23</i>	1,5826	0,2384	0,2613	0,0299
AtCg00350	<i>psaA</i>	0,4837	0,2721	0,2099	0,0715	AtCg00860	<i>ycf2.1</i>	2,2036	0,2554	0,0595	0,0528
AtCg00360	<i>ycf3</i>	2,3968	0,2409	0,3084	0,0205	AtCg00870	<i>ycf15</i>	2,1766	0,2122	-0,2576	0,0420
AtCg00380	<i>rpS4</i>	0,9036	0,1102	-0,0262	0,0381	AtCg00890	<i>ndhB</i>	2,1289	0,3321	0,1418	0,0845
AtCg00420	<i>ndhJ</i>	1,1838	0,0364	0,0333	0,0604	AtCg00900	<i>rpS7</i>	1,6901	0,2161	-0,0088	0,0251
AtCg00430	<i>ndhK</i>	1,1638	0,1312	0,0712	0,0474	AtCg01000	<i>ycf1</i>	1,8017	0,0899	1,0360	0,0046
AtCg00440	<i>ndhC</i>	1,2007	0,1800	0,0883	0,0377	AtCg01010	<i>ndhF</i>	1,6878	0,1986	0,4700	0,0301
AtCg00470	<i>atpE</i>	1,3050	0,3040	0,1823	0,0312	AtCg01020	<i>rpL32</i>	1,4453	0,1131	0,5855	0,2508
AtCg00480	<i>atpB</i>	0,6658	0,1833	0,2143	0,0243	AtCg01040	<i>ccsA</i>	1,2967	0,1533	0,4215	0,0332
AtCg00490	<i>rbcL</i>	-0,0113	0,2145	0,4040	0,0365	AtCg01050	<i>ndhD</i>	1,5050	0,4549	-0,0615	0,0477
AtCg00500	<i>accD</i>	1,8685	0,6108	-0,3235	0,0984	AtCg01060	<i>psaC</i>	1,0875	0,3100	0,2204	0,0920
AtCg00510	<i>psaI</i>	1,0765	0,2086	0,0628	0,0176	AtCg01070	<i>ndhE</i>	1,6530	0,2138	0,0411	0,0742
AtCg00520	<i>ycf4</i>	1,5718	0,1285	0,1478	0,0344	AtCg01080	<i>ndhG</i>	2,1465	0,1331	0,0446	0,1402
AtCg00530	<i>cemA</i>	1,7827	0,3528	-0,1010	0,0484	AtCg01090	<i>ndhI</i>	1,6501	0,0897	0,1128	0,1466
AtCg00540	<i>petA</i>	1,8801	0,0684	-0,0745	0,0203	AtCg01100	<i>ndhA</i>	1,2454	0,2785	0,3119	0,0342
AtCg00550	<i>psbJ</i>	2,2268	0,2562	0,0380	0,0620	AtCg01110	<i>ndhH</i>	1,4940	0,2229	0,1077	0,0572
AtCg00560	<i>psbL</i>	1,4054	0,1798	0,2330	0,0409	AtCg01120	<i>rpS15</i>	2,1629	0,1058	0,1773	0,1285
AtCg00570	<i>psbF</i>	1,4384	0,1856	0,2257	0,0386	AtCg00920	<i>rrn16S</i>	0,9788	0,2123	0,2818	0,2123
AtCg00580	<i>psbE</i>	1,3913	0,1572	0,1761	0,1059	AtCg00950	<i>rrn23S</i>	3,1049	0,1752	0,0527	0,1752
AtCg00590	<i>petL</i>	1,6698	0,1699	0,1909	0,0140	18S rRNA	<i>nuc18S</i>	0,4364	0,2150	-0,1760	0,2150
AtCg00600	<i>petG</i>	1,7946	0,1898	0,2499	0,0376	At5g25760	<i>UBC</i>	-0,2292	0,2230	-0,0954	0,0755
AtCg00630	<i>psaJ</i>	1,5379	0,1534	0,6279	0,0287	At5g08290	<i>YLS8</i>	-0,1251	0,1547	-0,0264	0,2767
AtCg00640	<i>rpL33</i>	1,5574	0,1316	0,1686	0,1864	At3g18780 & At1g49240	<i>actin2.8</i>	0,0390	0,1198	0,2592	0,1092

**Supplemental Table 4**

Frequencies of CGN codons in *Arabidopsis* chloroplast-encoded protein genes

Codon	cp-coded proteins 162 CDS's (41837 codons)			ribosomal proteins 29 CDS's (4008 codons)			rpo 4 CDS's (3461 codons)			Psa 5 CDS's (1651 codons)		
	#	%Total	%CGN	#	%Total	%CGN	#	%Total	%CGN	#	%Total	%CGN
CGG	192	0,5	14,3	28	0,7	11,0	27	0,8	18,1	1	0,1	2,7
CGA	461	1,1	34,5	99	2,5	39,0	66	1,9	44,3	12	0,7	32,4
CGU	516	1,2	38,6	103	2,6	40,6	32	0,9	21,5	19	1,2	51,4
CGC	169	0,4	12,6	24	0,6	9,4	24	0,7	16,1	5	0,3	13,5
Total	1338			254			149			37		

Codon	Psb 15 CDS's (2407 codons)			Rbc1 1 CDS (480 codons)			PetA 1 CDS (321 codons)			PetB 1 CDS (216 codons)		
	#	%Total	%CGN	#	%Total	%CGN	#	%Total	%CGN	#	%Total	%CGN
CGG	5	0,2	6,6	0	0,0	0,0	0	0,0	0,0	0	0,0	0,0
CGA	18	0,7	23,7	6	1,3	30,0	3	0,9	33,3	2	0,9	22,2
CGU	43	1,8	56,6	12	2,5	60,0	4	1,2	44,4	5	2,3	55,6
CGC	10	0,4	13,2	2	0,4	10,0	2	0,6	22,2	2	0,9	22,2
Total	76			20			9			9		

### Supplemental Table 5

Primers used in this study

Name	Purpose	Sequence
P1	Genotyping	CGAGTTTATCTGAAGAAGCCC
P2	GFP construction	CAAGGTACCTCTTCTAATTCAAC
P3	Genotyping	TCCACCCACAAGTAACCTCC
P4	GFP construction	CTTGGATCCACTTCTCTTTCTTC
P5	Genotyping	GGAGACTCGTTATAGCAGTGAG
P6	Genotyping	GCCTCTTCAGATAGCTTTGACTG
P7	TADA RT-PCR	AGGTGGAAACTTTGAGTTCC
P8	$\Delta$ N-TADA pRSET cloning	GCGGATCCACTAGTCATGAGGGAAGCGTTAG
P9	TADA RT-PCR	CTGCATGGGCAGTTGAATCACG
P10	$\Delta$ N-TADA pRSET cloning	AGGAATTCTACAGACAGAAAAGG
P11	cp-trnR editing test	GGGCTTGTAGCTCAGAGG
P12	cp-trnR editing test	TGGTGGGCGAGGAGGGATTCC
P13	trnA editing test	GGGGATGTAGCTCAGATGGTAG
P14	trnA editing test	TGGTGGAGATGCGGGGTATC
P15	trnV editing test	GGTTTCGTGGTGTAGTTGG
P16	trnV editing test	TGGTGGCTTCGCCCCGGTTC
P17	trnT editing test	GCTTTCATAGCTCAGTTGG
P18	trnT editing test	TGGTGCTTTCGTTGAGAGTTG
P19	trnL editing test	GTTGATATGGCCGAGTTGG
P20	trnL editing test	TGGTGTGACAGTGGGATTTC
P21	trnI editing test	GGCCATTAGCTCAGTTGG
P22	trnI editing test	TGGTGGCCCATGCAGG
P23	trnR editing test	GACTCCATGGCCCAATG
P24	trnR editing test	TGGCTGGGGATCGAACC
P25	ndhB RT-PCR	CAACTCTGTATTCTCTATCC
P26	ndhB RT-PCR	AGCTAGGATTTCCAGAAGAAGATGC
P27	ndhD RT-PCR	GCGGCTTTTCCAGTTACTCGGGATTCC
P28	ndhD RT-PCR	CAGGATCCGTTATAGAATAATACC
P29	rps12 RT-PCR	TCTCACACCGGGTAAATCCT
P30	rps12 RT-PCR	ATCCGAAACGTCACGAAATC
P31	rps14 RT-PCR	ATAGGGAGAAGAAGAGGC
P32	rps14 RT-PCR	TGAACCATTTCCCGAAGG
P33	nad3 RT-PCR	AGCGAGAAAACAAAGTGGGC
P34	nad3 RT-PCR	TGCCCTATCACTTTACTCCC
P35	nad5 RT-PCR	GGTTACATCCATAAGTAGCTTGGTCC
P36	nad5 RT-PCR	GCGATGACCCTCTTTAGATCG
P37	cox3 RT-PCR	ATACCATGTTCTGTATGGTGG
P38	cox3 RT-PCR	AAACCATGAAAGCCTGTTGC
P39	ccb206 RT-PCR	CTCGTATATCGTGTAAACGC
P40	ccb206 RT-PCR	TGGATTTTGCAGCTGTTCC
P41	ccmC RT-PCR	TCAAAGACCAGAAGCTACGC
P42	ccmC RT-PCR	CAAGATACGGGTTGAGAAGG
P43	ccmFc RT-PCR	TTTATGGTCTGTGCTTGTGG
P44	ccmFc RT-PCR	TTTCGTCTGTGCTCATTCCC
P45	rps12 RT-PCR	GAAAAGAGAATAATGCCACG
P46	rps12 RT-PCR	GAGGCATCTTCCATTCATATCG
P47	$\Delta$ N-TADA construction	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGGTCTCCTGCTGTAATGG
P48	$\Delta$ N-TADA construction	GGGGACCACTTTGTACAAAGAAAGCTGGGTCTTCCATTGTGTGGAGGA
P49	$\Delta$ N-TADA genotyping	GTTCGAAGGTGAAGGTGACG
P50	$\Delta$ N-TADA genotyping	CTGCATGGGCAGTTGAATCACG
P51	$\Delta$ N-TADA genotyping	TCATGAGGGAAGCGTTAG
P52	$\Delta$ N-TADA genotyping	GCGGGATATCACCACCTTTGTA
P53	cp-trnR in vitro synthesis	GAGGATCCGAATTGTAATACGACTCACTATAGGGCTTGTAGCTCAGAG
P54	cp-trnR in vitro synthesis	GAGAATTCCTGGTGGGCGAGGAGGGATTCC
P55	trnR in vitro synthesis	GAGTAATACGACTCACTATAGGACTCCATGGCCCAATGG
P56	trnR in vitro synthesis	CTGGATCCTGGCGACTCCACTGGGGATCGAACCAG
P57	trnA in vitro synthesis	GAGTAATACGACTCACTATAGGGGATGTAGCTCAGATGG
P58	trnA in vitro synthesis	CTGGATCCTGGTGGAGATGCGGGGTATCG
actin2.8F	qRT-PCR	GGTAACATTGTGCTCAGTGGTGG
actin2.8R	qRT-PCR	AACGACCTTAATCTTCATGCTGC
Q18SF	qRT-PCR	AAACGGCTACCACATCCAAG
Q18SR	qRT-PCR	ACTCGAAAAGAGCCCGGTATT
UBC-1	qRT-PCR	TTCGTTCTTTGGGAAATTAGA
UBC-2	qRT-PCR	CTCGCTGTACCTCTTTGATTCTTT
YLS8-1	qRT-PCR	GGGATGAGACCTGTATGCAGATGGA
YLS8-2	qRT-PCR	GCTCGTACATGGTGTGAAGTCTGG