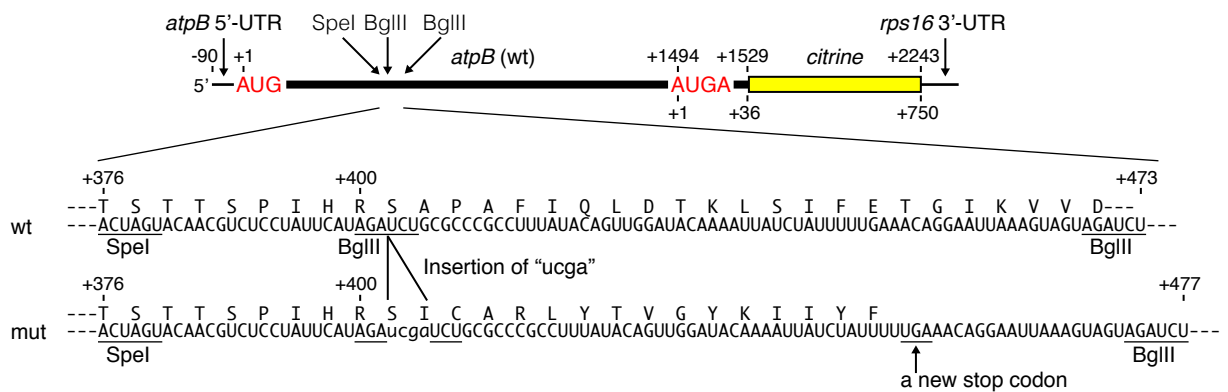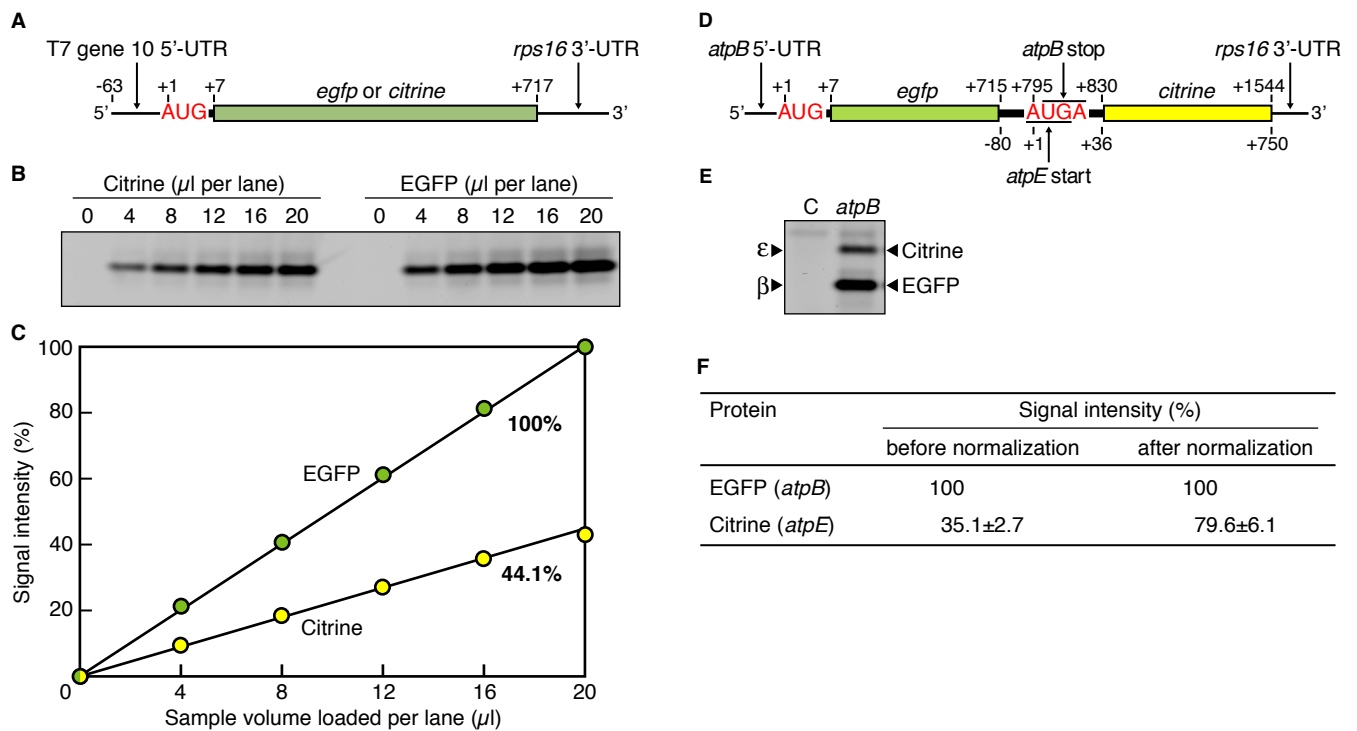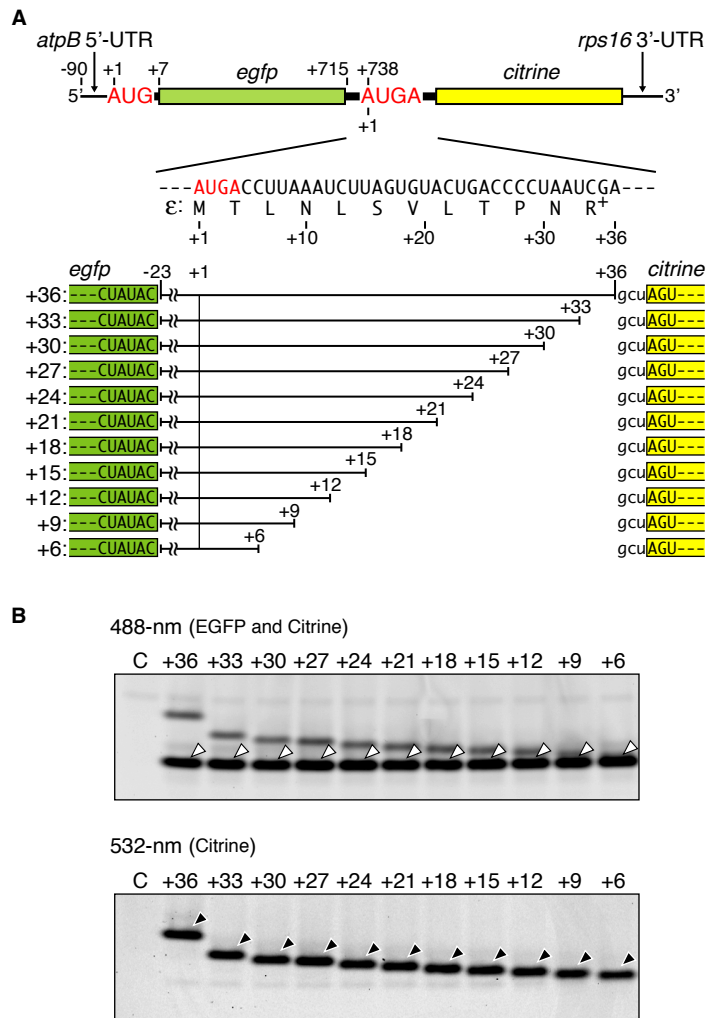**Supplementary Figure S1.** A flow chart of plasmid construction. Filled box, T7 promoter; green box, *egfp*; yellow box, *citrine*; red box, *atpB/E* portions; white box, PCR products and annealed oligonucleotides. Oligonucleotides used in the numbered steps are listed in Supplementary Figure S9.

atpB 5'-UTR    SpeI BgIII  BgIII                                    rps16 3'-UTR

-90 | +1          ↓ ↓        atpB (wt)      +1494  +1529   citrine   +2243
5' — AUG                                    AUGA                        ↓
                                            +1     +36              +750

+376                    +400                                              +473
    T  S  T  T  S  P  I  H  R  S  A  P  A  F  I  Q  L  D  T  K  L  S  I  F  E  T  G  I  K  V  V  D
wt  ---ACUAGUACAACGUCUCCUAUUCAUAGAUCUGCGCCCGCCUUUAUACAGUUGGAUACAAAAUUAUCUAUUUUUGAAACAGGAAUUAAAGUAGUAGAUCU---
       SpeI                  BgIII                                                              BgIII
                                   \  Insertion of "ucga"
+376                    +400                                              +477
    T  S  T  T  S  P  I  H  R  S  I  C  A  R  L  Y  T  V  G  Y  K  I  I  Y  F
mut ---ACUAGUACAACGUCUCCUAUUCAUAGAucgaUCUGCGCCCGCCUUUAUACAGUUGGAUACAAAAUUAUCUAUUUUUGAAACAGGAAUUAAAGUAGUAGAUCU---
       SpeI                                                            ↑                        BgIII
                                                                  a new stop codon

**Supplementary Figure S2.** A frameshift mutation in the *atpB* cistron. Sequences between SpeI/BgIII sites. There are two BgIII sites, and "ucga" was inserted into the upstream BgIII site. Deduced amino acid sequences are shown above RNA sequences. A new stop codon in the mutant (mut) is also indicated.
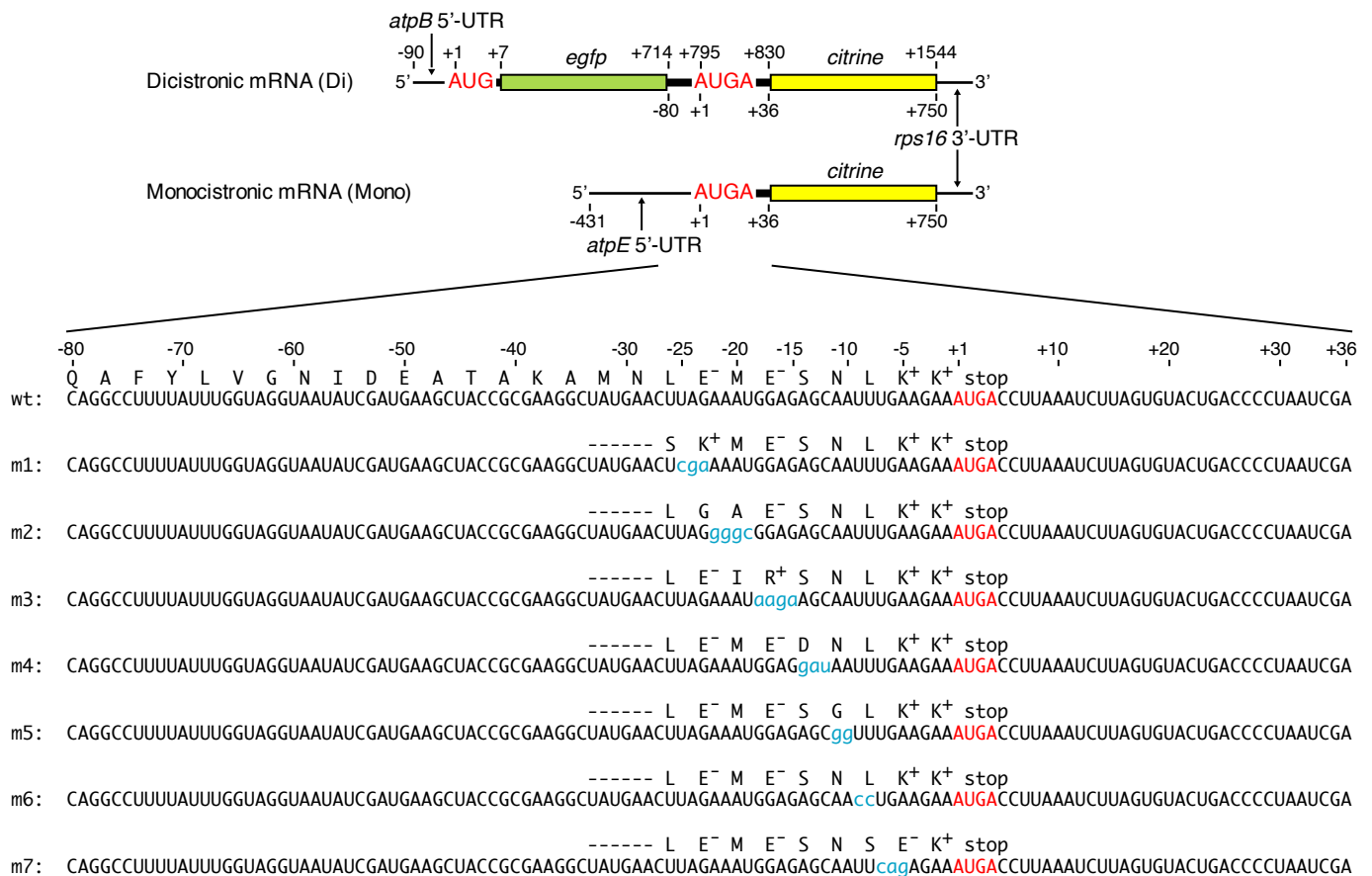
**Supplementary Figure S3.** The full mRNA sequence in Figure 2. T7 RNA polymerase generally starts to synthesize RNAs from pppGGG. Hence, the 5'-end has two additional Gs before G (-90) in the dicistronic mRNA and one additional G in front of G (-431) in the monocistronic mRNA. Lower cases are restriction sites used for ligation.

**Supplementary Figure S4.** Comparison of fluorescence intensities of EGFP and Citrine. **(A)** Schematic of test mRNAs. **(B)** Gel patterns of EGFP and Citrine products (both 239 aa) translated from the same T7 gene 10 5'-UTR for 1 h at 28 °C. Fluorescent signals were detected by irradiation of a 488-nm actinic light with a 520BP40 filter. Indicated volumes (µl) of reaction mixtures were loaded. **(C)** Quantified fluorescent signals (calibration curves). Fluorescence of the Citrine was about 44.1% of that of the EGFP by 488-nm excitation. **(D)** Test mRNA in Figure 3. **(E)** Gel patterns of synthesized EGFP (from *atpB*) and Citrine (from *atpE*) products shown in Figure 3. Lane C, no mRNA added as control. **(F)** Quantification of fluorescence intensities of EGFP and Citrine by 488-nm excitation. The raw value was normalized based on the calibration curves (C).

5'-UTR

+1 +7 *egfp* +715 +795 +830 *rps16* 3'-UTR

5' AUG █ ════════════ █ AUGA ▓▓▓▓▓▓▓▓▓▓ 3'
                                    *citrine*
                        -80 +1 +36        +750

*atpE* -80/+36

-90                                                                                    +1
*atpB* 5'-UTR   pppGG|GAAUUAACCGAUCGACGUGCUAGCGGACAUUUAUUUUGAAUUCGAUAAUUUUUGCAAAAACAUUUCGACAUAUUUAUUUAUUUUAUUAUU|AUG

+7
*egfp* cistron   gcu|AGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUA
CGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCUUGGCCAACACUUGUCACUACUUUAACUUAUGGUGUUCAAUGCUUUUCAAGGUACCCAGAUCAUA
UGAAACAACAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGU
GAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGA
CAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCC
UUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGUCCUUCUUGAGUUUGUAACAGCUGCUGGGAUUACACUUGGC
AUGGAUGAACUAUAC|

+715        A↑                                                                         +795                        +830
*atpE*-80/+36   |CAGGCCUUUUAUUUGGUAGGUAAUAUCGAUGAAGCUACCGCGAAGGCUAUGAACUUAGAAAUGGAGAGCAAUUUGAAGAA**AUGA**CCUUAAAUCUUAGUGUACUGACCCCUAAUCGA|
                -80        -69                                                           +1                               +36

*citrine* cistron   gcu|AGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUA
CGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCUUGGCCAACACUUGUCACUACUUUUGGAUAUGGUUUAAAUGUGCUUUGCUAGGUACCCAGAUCAUA
UGAAACAACAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGU
GAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGA
CAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCC
UUUUUACCAGACAACCAUUACCUGUCCUAUCAAUCUGCCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGUCCUUCUUGAGUUUGUAACAGCUGCUGGGAUUACACUUGGC
AUGGAUGAACUAUACAAA|UAA

-750
*rps16* 3'-UTR   cuacagauaaaaagaucuuaaucuagc|GAAAUUCAAUUAAGGAAAUAAAAUUAAGGAAAUACAAAAAGGGGGUAGUCAUUUGUAUAUAACUUUGUAUGACUUUUCUCUUCUAUUUU
UUUGUAUUUCCUCCCUUUCCUUUUUCUAUUUGUAUUUUUUUUAUCAUUGCUUCCAUUGAAUUC|ucga$_{OH}$

---

*atpH* 5'-UTR + AUG

-45                                                          +1      +7 *egfp*
5'-pppGGG|AUUGUAUCAUUAACCAUUUCUUUUUUUUUGGUACGAGGAACUUAUC|AUGgcu|AGU---|

---

*rbcL* 5'-UTR + AUG

+59                                                              +1      +7 *egfp*
5'-pppGGG|UGUCGAGUAGACCUUGUUGUUGUGAGAAUUCUUAAUUCAUGAGUUGUAGGGAGGGAUUU|AUGgcu|AGU---|

---

No 5'-UTR nor AUG

*egfp*
5'-pppGGGCU|AGU---|

---

**Supplementary Figure S5.** The full mRNA sequence in Figure 3 and other 5'-UTR sequences. Since test mRNAs were synthesized with T7 RNA polymerase, their 5'-ends were expected to start with an extension of two or three Gs. The "gcu" in front of *egfp* and *citrine* is a result of ligation between an *Nhe*I-digested PCR product and an *Spe*I-digested *egfp* or *citrine*. Small letters between *citrine* and *rps16* 3'-UTR are a linker sequence. A point mutation (U to A) at -69 relative to an A (+1) of the *atpE* start codon in Figure 4A is marked.

**Supplementary Figure S6.** Effect of internal deletions in the 5'-*atpE* coding region on translation of the *atpE* cistron. **(A)** The mRNA as shown in Figure 3A and a sequence of the 5'-*atpE* coding region (12 codons). mRNAs with internal deletions (indicated as blanks, from +7 to +36) are shown below. Terminal sequences of *egfp* and *citrine* are boxed. **(B)** Gel patterns of synthesized EGFP and Citrine products. White arrowheads in the upper panel and black arrowheads in the lower panel point EGFP and Citrine bands, respectively.
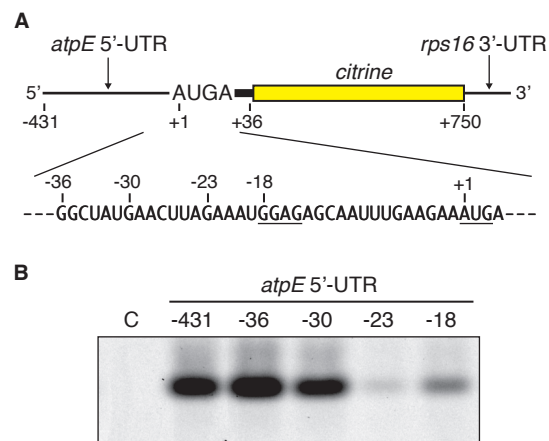
**Supplementary Figure S7.** Effect of 5' deletions of the monocistronic *atpE* mRNA on *atpE* translation. **(A)** The mRNA as in Figure 2A and a partial *atpE* 5'-UTR sequence. The SD-like sequence and the start codon are underlined. **(B)** Gel pattern of synthesized Citrine products. Translation products were detected by irradiation with 532-nm light.

**Supplementary Figure S8.** Test mRNAs in Figure 7. Sequences surrounding the *atpE* start codon. Blue lower cases indicate mutagenized nucleotides. Deduced amino acid sequences from wild type (wt) and mutant (m1 to m7) 3'-*atpB* cistrons are shown above the nucleotide sequences.

**Supplementary Figure S9.** Lists of oligonucleotides used in this study.

**Supplementary Figure S1:**
Step #1: On-1 and On-2
Step #2: On-3 and On-4
Step #3: On-5 and On-6
Step #4: On-7, On-8 and On-9
Step #5: On-10 and On-11
Step #6: On-5 and On-12
Step #7: On-13 and On-14
Step #8: On-15 and On-16
Step #9: On-13 and On-17
Step #10: On-17 and On-18
Step #11: On-17 and On-19

**Figure 3:**
*atpH* 5'-UTR/AUG: On-20 and On-21
*rbcL* 5'-UTR/AUG: On-22 and On-23
No 5'-UTR/AUG: On-24 and On-25

**Figure 4:**
Point mutation at "-69" to created a new stop codon: On-26 and On-27

**Figure 5:**
Mutation at the BglII site in the *atpB* cistron to create a new stop codon: On-28 and On-29

**Figure 6:**
"-29": On-17 and On-30
"-26": On-17 and On-31
"-23": On-17 and On-32
"-20": On-17 and On-33
"-17": On-17 and On-34
"-14": On-17 and On-35
"-11": On-17 and On-36
"-8": On-17 and On-37
"-5": On-17 and On-38
"-2": On-17 and On-39

**Figure 7:**
"m1": On-17, On-40 and On-41
"m2": On-17, On-41 and On-42
"m3": On-17, On-41 and On-43
"m4": On-17, On-41 and On-44
"m5": On-17, On-41 and On-45
"m6": On-17, On-41 and On-46
"m7": On-17, On-41 and On-47

**Supplementary Figure S6:**
"+36": On-17 and On-32
"+33": On-32 and On-48
"+30": On-32 and On-49
"+27": On-32 and On-50

"+24": On-32 and On-51
"+21": On-32 and On-52
"+18": On-53 and On-54
"+15": On-55 and On-56
"+12": On-57 and On-58
"+9": On-59 and On-60
"+6": On-61 and On-62


On-1: 5'-AACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGC
On-2: 5'-TCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCC
On-3: 5'-CCTTTCGAAAGATCCCAACGAAAG
On-4: 5'-CCCTCTAGATTATACGTATAGTTCATCCATGCCAAGTGTAATCCCAG
On-5: 5'-GGGCCATGGCTACTAGTAAAGGAGAAGAACTTTTCACTGGAG
On-6: 5'-GGGGTACCTAGCAAAGCATTGTAAACCATATCCAAAAG
On-7: 5'-CAGATCATATGAAACAACATGACTTTTTCA
On-8: 5'-TCTTTCGAAAGGGCAGATTGATAGGACAGGTAATGGTTGTCTG
On-9: 5'-GAGCGGATAACAATTTCACACAGG
On-10: 5'-AATTCTCGAGTCGACGATATC
On-11: 5'-AATTGATATCGTCGACTCGAG
On-12: 5'-CTGGGTACCTAGCAAAGCACATTAAACCATATCCAAAAG
On-13: 5'-GGGCTAGCCATAATAATAAAATAAATAAAT
On-14: 5'-CCATAATAATAAAATAAATAAATATGTCGAAATG
On-15: 5'-AAATAACTACAGATAAAAGATCTTAAT
On-16: 5'-CTAGATTAAGATCTTTTTATCTGTAGTTATTT
On-17: 5'-GGGCTAGCTCGATTAGGGGTCAGTACACTA
On-18: 5'-GATTGGCTGCCAAAGGTATTTATCC
On-19: 5'-CAGGCCTTTTATTTGGTAGGTAATATCG
On-20: 5'-GATTGTATCATTAACCATTTCTTTTTTTTG
On-21: 5'-GGGCTAGCCATGATAAGTTCCTCGTACCAA
On-22: 5'-GTGTCGAGTAGACCTTGTTGTTG
On-23: 5'-GGGCTAGCCATAAATCCCTCCCTACAACTC
On-24: 5'-AGCTTGCTGTAATACGACTCACTATAGGG
On-25: 5'-CTAGCCCTATAGTGAGTCGTATTACAGCA
On-26: 5'-CCTTTTAATTGGTAGGTAATAT
On-27: 5'-CGATATTACCTACCAATTAAAAGG
On-28: 5'-ATACTAGTACAACGTCTCCTATTCATAGATCGATCTGCGCCCGCCTTTAT
On-29: 5'-CATTTCCTTCCCGAGTACGTTCACCC
On-30: 5'-AACTTAGAAATGGAGAGCAATTTGAAG
On-31: 5'-TTAGAAATGGAGAGCAATTTGAAGAAATGA
On-32: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCT
On-33: 5'-ATGGAGAGCAATTTGAAGAAATGACCTTAA
On-34: 5'-GAGAGCAATTTGAAGAAATGACCTTAAATC
On-35: 5'-AGCAATTTGAAGAAATGACCTTAAATCTTA
On-36: 5'-AATTTGAAGAAATGACCTTAAATCTTAGTG
On-37: 5'-TTGAAGAAATGACCTTAAATCTTAGTG
On-38: 5'-AAGAAATGACCTTAAATCTTAGTGTACTG
On-39: 5'-AAATGACCTTAAATCTTAGTGTACTG
On-40: 5'-AGGCTATGAACTCGAAAATGGAGAGCAATT
On-41: 5'-CGCCAGGGTTTTCCCAGTCACGAC
On-42: 5'-GCTATGAACTTAGGGGCGGAGAGCAATTTG

On-43: 5'-GGCTATGAACTTAGAAATAAGAAGCAATTTGAAGAAATGACC
On-44: 5'-CTATGAACTTAGAAATGGAGGATAATTTGAAGAAATGACCTTAAATC
On-45: 5'-AGAAATGGAGAGCGGTTTGAAGAAATGACC
On-46: 5'-GAAATGGAGAGCAACCTGAAGAAATGACCT
On-47: 5'-CTTAGAAATGGAGAGCAATTCAGAGAAATGACCTTAAATCTTAGTG
On-48: 5'-GGGCTAGCATTAGGGGTCAGTACACTAAGATTT
On-49: 5'-GGGTCTAGAGCTAGCAGGGGTCAGTACACTAAGATTTAAG
On-50: 5'-GGGCTAGCGGTCAGTACACTAAGATTTAAGGTC
On-51: 5'-GGGCTAGCCAGTACACTAAGATTTAAGGTCATTTC
On-52: 5'-GGGCTAGCTACACTAAGATTTAAGGTCATTTCTTC
On-53: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCTTAAATCTTAGTG
On-54: 5'-CTAGCACTAAGATTTAAGGTCATTTCTTCAAATTGCTCTCCATTTC
On-55: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCTTAAATCTTG
On-56: 5'-CTAGCAAGATTTAAGGTCATTTCTTCAAATTGCTCTCCATTTC
On-57: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCTTAAATG
On-58: 5'-CTAGCATTTAAGGTCATTTCTTCAAATTGCTCTCCATTTC
On-59: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCTTAG
On-60: 5'-CTAGCTAAGGTCATTTCTTCAAATTGCTCTCCATTTC
On-61: 5'-GAAATGGAGAGCAATTTGAAGAAATGACCG
On-62: 5'-CTAGCGGTCATTTCTTCAAATTGCTCTCCATTTC