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***Research article***

**The expression level and cytotoxicity of green fluorescent protein are modulated by an additional N-terminal sequence**

**Hisao Moriya\***

Graduate School of Environmental and Life Sciences, Research Core for Interdisciplinary Sciences, Okayama University

**\* Correspondence:** Email: [hisaom@cc.okayama-u.ac.jp](mailto:hisaom@cc.okayama-u.ac.jp); Tel: +81862518712.

*Appendix*

**Table S1.** Raw data of measurement.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Clone# | MGR–Ura | Copy#–Ura | GFP–Ura | MGR−Leu/Ura | Copy#−Leu/Ura (CNL) | GFP−Leu/Ura | GFP-Ura/copy#–Ura (EPG) |
| 1 | 5.8 | 49.5 | 1006 | 3.9 | 73.0 | 1110 | 20 |
| 2 | 5.4 | 33.6 | 2332 | 2.6 | 36.5 | 1383 | 69 |
| 3 | 4.3 | 23.6 | 2330 | 1.5 | 25.6 | 1315 | 99 |
| 4 | 5.3 | 66.7 | 329 | 3.2 | 98.4 | 200 | 5 |
| 5 | 5.2 | 18.3 | 2169 | 2.2 | 28.8 | 1371 | 119 |
| 6 | 4.6 | 20.5 | 2855 | 1.2 | 31.6 | 855 | 139 |
| 7 | 4.6 | 25.8 | 2030 | 1.3 | 38.1 | 1002 | 79 |
| 8 | 4.9 | 22.2 | 1887 | 1.4 | 26.0 | 1447 | 85 |
| 9 | 4.4 | 21.9 | 1799 | 1.6 | 34.1 | 1101 | 82 |
| 10 | 4.3 | 31.3 | 2325 | 1.2 | 43.4 | 936 | 74 |
| 11 | 4.9 | 14.7 | 1686 | 1.8 | 22.8 | 1061 | 115 |
| 12 | 5.3 | 34.5 | 1221 | 3.2 | 68.1 | 1604 | 35 |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Clone# | MGR–Ura | Copy#–Ura | GFP–Ura | MGR−Leu/Ura | Copy#−Leu/Ura (CNL) | GFP−Leu/Ura | GFP-Ura/copy#–Ura (EPG) |
| 13 | 5.1 | 9.0 | 1279 | 2.1 | 29.7 | 1083 | 142 |
| 14 | 5.3 | 35.3 | 758 | 2.6 | 66.7 | 662 | 21 |
| 15 | 3.2 | 10.2 | 1197 | 0.8 | 12.7 | 27 | 117 |
| 16 | 4.8 | 15.1 | 1057 | 2.2 | 34.5 | 746 | 70 |
| 17 | 5.3 | 44.3 | 1239 | 3.0 | 74.0 | 1346 | 28 |
| 18 | 2.9 | 7.5 | 435 | 0.6 | 15.5 | 72 | 58 |
| 19 | 6.1 | 30.7 | 1699 | 2.9 | 47.8 | 1492 | 55 |
| 20 | 5.0 | 30.7 | 2375 | 2.1 | 41.6 | 1365 | 77 |
| 21 | 5.6 | 49.9 | 672 | 2.5 | 93.1 | 693 | 13 |
| 22 | 4.3 | 8.9 | 995 | 0.6 | 15.0 | 51 | 112 |
| 23 | 4.7 | 28.2 | 2265 | 1.5 | 27.9 | 1258 | 80 |
| 24 | 5.9 | 34.5 | 956 | 2.7 | 61.4 | 826 | 28 |
| 25 | 4.6 | 35.5 | 2220 | 1.9 | 42.5 | 1354 | 63 |
| 26 | 4.2 | 23.6 | 1822 | 1.1 | 23.6 | 650 | 77 |
| 27 | 4.5 | 23.8 | 1931 | 1.3 | 29.7 | 873 | 81 |
| 28 | 4.9 | 28.1 | 2006 | 1.9 | 39.7 | 617 | 72 |
| 29 | 5.2 | 25.3 | 1578 | 2.4 | 45.6 | 1037 | 62 |
| 30 | 3.6 | 13.3 | 1488 | 0.8 | 28.4 | 935 | 112 |
| 31 | 4.3 | 28.4 | 2323 | 1.4 | 34.1 | 1227 | 82 |
| 32 | 6.2 | 37.5 | 877 | 2.5 | 71.0 | 761 | 23 |
| 33 | 5.5 | 44.3 | 1401 | 2.2 | 64.9 | 1263 | 32 |
| 34 | 5.9 | 45.6 | 1542 | 3.4 | 59.7 | 1207 | 34 |
| 35 | 3.7 | 10.7 | 1143 | 1.2 | 19.0 | 37 | 107 |
| 36 | 6.2 | 58.5 | 864 | 3.7 | 96.3 | 1056 | 15 |
| 37 | 4.9 | 22.0 | 1983 | 1.8 | 34.1 | 842 | 90 |
| 38 | 4.5 | 19.8 | 2123 | 1.3 | 29.7 | 1116 | 107 |
| 39 | 6.0 | 45.6 | 1692 | 3.5 | 75.6 | 1657 | 37 |
| 40 | 4.7 | 15.3 | 1923 | 1.4 | 23.8 | 959 | 125 |
| 41 | 4.0 | 14.8 | 2237 | 1.3 | 26.0 | 1344 | 151 |
| 42 | 4.2 | 18.3 | 2047 | 1.1 | 30.3 | 862 | 112 |
| 43 | 4.4 | 30.1 | 2045 | 1.2 | 33.4 | 1053 | 68 |

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|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Clone# | MGR–Ura | Copy#–Ura | GFP–Ura | MGR−Leu/Ura | Copy#−Leu/Ura (CNL) | GFP−Leu/Ura | GFP-Ura/copy#–Ura (EPG) |
| 44 | 4.0 | 21.3 | 2592 | 1.2 | 33.4 | 991 | 122 |
| 45 | 5.0 | 17.9 | 1343 | 2.0 | 30.3 | 425 | 75 |
| 46 | 5.2 | 30.3 | 1921 | 2.5 | 39.9 | 1187 | 63 |
| 47 | 4.0 | 27.3 | 2366 | 1.6 | 31.1 | 974 | 87 |
| 48 | 5.3 | 18.0 | 2008 | 2.2 | 34.8 | 1385 | 112 |
| 49 | 3.4 | 3.6 | 10 | 0.7 | 13.7 | 15 | 3 |
| 50 | 4.7 | 35.3 | 1542 | 1.5 | 45.6 | 943 | 44 |
| 51 | 3.3 | 3.3 | 11 | 0.6 | 8.9 | 14 | 3 |
| 52 | 3.0 | 5.2 | 9 | 0.5 | 15.0 | 15 | 2 |
| 53 | 2.7 | 5.1 | 11 | 0.6 | 17.5 | 14 | 2 |
| 54 | 4.5 | 14.0 | 8 | 1.6 | 56.9 | 15 | 1 |
| 55 | 6.1 | 62.2 | 10 | 3.6 | 90.5 | 15 | 0 |
| 57 | 5.5 | 42.5 | 29 | 3.2 | 74.5 | 26 | 1 |
| 58 | 3.9 | 12.6 | 471 | 1.1 | 25.8 | 99 | 37 |
| 59 | 5.8 | 37.3 | 1357 | 3.1 | 74.5 | 1034 | 36 |
| 60 | 6.2 | 54.2 | 556 | 3.9 | 99.7 | 758 | 10 |
| 61 | 5.3 | 37.8 | 139 | 2.9 | 59.7 | 105 | 4 |
| 62 | 4.1 | 36.5 | 8 | 3.1 | 66.3 | 14 | 0 |
| 63 | 3.1 | 3.9 | 10 | 0.7 | 13.4 | 14 | 3 |
| 64 | 2.9 | 3.6 | 10 | 0.4 | 13.9 | 14 | 3 |
| 65 | 4.3 | 9.8 | 946 | 0.5 | 15.5 | 153 | 96 |
| 66 | 4.2 | 42.2 | 1603 | 2.4 | 48.2 | 1538 | 38 |
| 67 | 5.7 | 49.9 | 527 | 3.6 | 101.1 | 640 | 11 |
| 68 | 4.7 | 19.6 | 187 | 2.5 | 44.3 | 194 | 10 |
| 69 | 3.8 | 3.9 | 553 | 0.5 | 28.6 | 18 | 142 |
| 70 | 5.7 | 49.5 | 74 | 3.1 | 113.0 | 46 | 1 |
| 71 | 5.8 | 55.3 | 247 | 3.1 | 132.5 | 272 | 4 |
| 72 | 5.9 | 48.5 | 383 | 2.7 | 100.4 | 254 | 8 |
| 73 | 3.8 | 15.9 | 984 | 1.6 | 35.3 | 306 | 62 |
| 74 | 6.0 | 59.3 | 13 | 3.8 | 85.6 | 31 | 0 |
| 75 | 2.8 | 4.3 | 454 | 0.7 | 6.1 | 15 | 107 |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Clone# | MGR–Ura | Copy#–Ura | GFP–Ura | MGR−Leu/Ura | Copy#−Leu/Ura (CNL) | GFP−Leu/Ura | GFP-Ura/copy#–Ura (EPG) |
| 76 | 6.1 | 46.5 | 379 | 3.7 | 71.5 | 261 | 8 |
| 77 | 5.4 | 56.5 | 1203 | 3.6 | 80.4 | 1503 | 21 |
| 78 | 3.6 | 16.9 | 1359 | 1.2 | 60.5 | 427 | 80 |
| 79 | 4.2 | 26.5 | 2163 | 2.2 | 47.8 | 1709 | 82 |
| 80 | 4.6 | 10.9 | 1187 | 0.1 | 13.5 | 156 | 109 |
| 82 | 3.6 | 6.6 | 592 | 0.1 | 12.3 | 16 | 89 |
| 83 | 4.1 | 17.5 | 2073 | 2.1 | 41.6 | 1017 | 118 |
| 84 | 5.8 | 24.1 | 1254 | 3.4 | 42.8 | 922 | 52 |
| 85 | 5.8 | 48.5 | 1139 | 4.0 | 86.8 | 1479 | 23 |
| 86 | 5.8 | 19.7 | 1267 | 3.3 | 32.9 | 1354 | 64 |
| 87 | 5.0 | 41.9 | 945 | 2.9 | 77.2 | 782 | 23 |
| 88 | 5.8 | 50.9 | 1183 | 2.7 | 93.1 | 989 | 23 |
| 89 | 4.1 | 28.2 | 1391 | 1.5 | 40.2 | 765 | 49 |
| 90 | 4.8 | 31.6 | 2367 | 2.0 | 52.7 | 1098 | 75 |
| 91 | 3.8 | 16.1 | 2156 | 1.2 | 30.1 | 1136 | 134 |
| 92 | 4.1 | 9.4 | 1304 | 0.1 | 23.9 | 191 | 139 |
| 93 | 4.1 | 26.9 | 1454 | 1.6 | 35.8 | 660 | 54 |
| 94 | 4.3 | 22.5 | 2232 | 2.8 | 24.4 | 1317 | 99 |
| 95 | 3.6 | 11.2 | 967 | 1.2 | 19.0 | 216 | 87 |
| 96 | 4.4 | 23.3 | 2453 | 1.2 | 30.7 | 1186 | 105 |

**Table S2.** Isolated N10 sequences and their characteristics.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clone # | | Category | | Nucleotide sequence | Comment | | | Amino acid sequence | mfold ΔG (kcal/mol) | | | tAI | | Localization (DeepLoc) | | Gravy Score | | N-terminal 30 a.a. | TMHMM v2.0 (first 30aa) | | | Jpred4 |
| #6 | | High nontoxic | | without N10 sequence | GFP | | |  |  | | |  | |  | |  | |  |  | | |  |
| #44 | | High nontoxic | | without N10 sequence | GFP | | |  |  | | |  | |  | |  | |  |  | | |  |
| #2 | | middle nontoxic | | ATGAGCATGATACGGGAGGTTGATGAATGTTTA |  | | | MSMIREVDECL | -4.3 | | | 0.187 | | Cytoplasm (0.8028) | | 0.11 | | MSMIREVDECLSKGEELFTGVVPILVELDG | noTM | | | alpha |
| #20 | | middle nontoxic | | ATGTGTACTGGACTCGATGTTGGCCCCGTCCAA |  | | | MCTGLDVGPVQ | -4.9 | | | 0.193 | | Cytoplasm (0.5431) | | 0.46 | | MCTGLDVGPVQSKGEELFTGVVPILVELDG | noTM | | | nd |
| #90 | | middle nontoxic | | ATGTCCGCAGCTGCGATCGGGTGCGGGTATGTA |  | | | MSAAAIGCGYV | -14.4 | | | 0.210 | | Cytoplasm (0.6262) | | 1.37 | | MSAAAIGCGYVSKGEELFTGVVPILVELDG | noTM | | | alpha |
| #1 | Low nontoxic | | ATGGGATGGCTGCCGAAAGGGTTCTTAGAAGAG | | |  | MGWLPKGFLEE | | | -6.5 | 0.307 | | Cytoplasm (0.6667) | | -0.38 | | MGWLPKGFLEESKGEELFTGVVPILVELDG | | | noTM | nd | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clone # | | | Category | | | Nucleotide sequence | Comment | | Amino acid sequence | | | | mfold ΔG (kcal/mol) | | | | tAI | | | Localization (DeepLoc) | | | | Gravy Score | | | N-terminal 30 a.a. | TMHMM v2.0 (first 30aa) | | | | Jpred4 | |
| #85 | Low nontoxic | | | ATGGTGGTGGTGGGTTGGCAGCCCCCGGGTGGG | | | |  | | | MVVVGWQPPGG | | | -8.3 | 0.210 | | | | Cytoplasm (0.8522) | | | 0.38 | | | | MVVVGWQPPGGSKGEELFTGVVPILVELDG | | | noTM | | beta | | |
| #15 | toxic | | | ATGTGCTTCGGGGTAATGAGGGGTATCTGGCTG | | | |  | | | MCFGVMRGIWL | | | -4.5 | 0.256 | | | | Mitochondrion (0.3977) | | | 1.35 | | | | MCFGVMRGIWLSKGEELFTGVVPILVELDG | | | noTM | | alpha/beta | | |
| #75 | | toxic | | | ATGTGCACTGAGCTGTGTTTTCTTCTCGGTGTC | | | | |  | | MCTELCFLLGV | | | | -8.4 | | 0.096 | | | Cytoplasm (0.5299) | | 1.88 | | MCTELCFLLGVSKGEELFTGVVPILVELDG | | | | | noTM | | | alpha/beta |
| #80 | | toxic | | | ATGCCGGTGATTGGGTATTTTGATGGCTTGTTA | | | | |  | | MPVIGYFDGLL | | | | -9.8 | | 0.242 | | | Cytoplasm (0.8578) | | 1.19 | | MPVIGYFDGLLSKGEELFTGVVPILVELDG | | | | | noTM | | | alpha |
| #82 | | toxic | | | ATGGATCCATTGTGCCAGTTGATGGGTGTGCTA | | | | |  | | MDPLCQLMGVL | | | | -6.4 | | 0.314 | | | Cytoplasm (0.7193) | | 1.10 | | MDPLCQLMGVLSKGEELFTGVVPILVELDG | | | | | noTM | | | alpha |
| #51 | | no\_expression toxic | | | witout N10 sequence, TDH3 | | | | | TDH3 | |  | | | |  | |  | | |  | |  | |  | | | | |  | | |  |
| #52 | | no\_expression toxic | | | witout N10 sequence, TDH3 | | | | | TDH3 | |  | | | |  | |  | | |  | |  | |  | | | | |  | | |  |
| #64 | | no\_expression toxic | | | witout N10 sequence, TDH3 | | | | | TDH3 | |  | | | |  | |  | | |  | |  | |  | | | | |  | | |  |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clone # | Category | Nucleotide sequence | Comment | Amino acid sequence | mfold ΔG (kcal/mol) | tAI | Localization (DeepLoc) | Gravy Score | N-terminal 30 a.a. | TMHMM v2.0 (first 30aa) | Jpred4 |
| #55 | no\_expression nontoxic | ATGTCCTCTGCTCATTCGTTGCCGGTATAGGGT | nonsense | MSSAHSLPV\*G | – | – |  |  |  |  |  |
| #74 | no\_expression nontoxic | ATGTAGAAATTTACGGTTGCGATCGCCAGAGTG | nonsense | M\*KFTVAIARV | – | – |  |  |  |  |  |
| #1\_opt |  | ATGGGTTGGTTGCCAAAGGGTTTCTTGGAAGAA |  | MGWLPKGFLEE | -5 | 0.426 |  |  | MGWLPKGFLEESKGEELFTGVVPILVELDG |  |  |
| #85\_opt |  | ATGGTTGTTGTTGGTTGGCAACCACCAGGTGGT |  | MVVVGWQPPGG | -11.7 | 0.384 |  |  | MVVVGWQPPGGSKGEELFTGVVPILVELDG |  |  |
| #15\_opt |  | ATGTGTTTCGGTGTTATGAGAGGTATCTGGTTG |  | MCFGVMRGIWL | -2.5 | 0.239 |  |  | MCFGVMRGIWLSKGEELFTGVVPILVELDG |  |  |
| #75\_opt |  | ATGTGTACCGAATTGTGTTTCTTGTTGGGTGTT |  | MCTELCFLLGV | -4.4 | 0.247 |  |  | MCTELCFLLGVSKGEELFTGVVPILVELDG |  |  |
| #80\_opt |  | ATGCCAGTTATCGGTTACTTCGACGGTTTGTTG |  | MPVIGYFDGLL | -4.9 | 0.371 |  |  | MPVIGYFDGLLSKGEELFTGVVPILVELDG |  |  |
| #82\_opt |  | ATGGACCCATTGTGTCAATTGATGGGTGTTTTG |  | MDPLCQLMGVL | -11.2 | 0.372 |  |  | MDPLCQLMGVLSKGEELFTGVVPILVELDG |  |  |

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