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***Research article***

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

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*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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