**Supporting Information Table 7**

**Predicted expression level of *patS* of *Anabaena* PCC 7120 from different start sites (arbitrary units)*a***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Strain*b*** | **Mutation*c*** | **M1 (ATG)** | **M5 (ATG)** | **V7 (GTG)** | **%Hets*b*** | **MCH*b*** |
|  | wt | 181 | 574 | 1446 | 10 | 4 |
| CSVT20 | ΔhetN | - - - | - - - | - - - | 19 | 21 |
| CSL44 | M1A | - - - | 9 | 43 | 19 | 26 |
| CSL49 | M5A | 161 | - - - | 806 | 11 | 7 |
| CSL93 | V7A*d* | 132 | 1437 | - - - | 3 | 0 |
| CSL51 | V7Q | 408 | 683 | - - - | 0 | 0 |
| CSL62 | M5V,V7Q*e* | 210 | 369 | - - - | 6 | 0 |
| a From Ribosome-Binding Site Calculator, (Salis and Mirsky, **2009**; https://salislab.net/software/doReverseRBS) using 40 nt upstream from M1 and the entire 54 nt of the *patS* gene.b From Corrales-Guerrero et al, (2013). The last two columns report the total number of heterocysts in the strain under nitrogen-fixing conditions and the percentage of multiple contiguous heterocysts.*c* Coordinates correspond to the system used by Corrales-Guerrero et al, (2013): M1 K2 A3 I4 M5 L6 V7 N8 F9 C10 D11 E12 R13 G14 S15 G16 R17 d Primer not given in article – presume mutation from GTG to GCG.e Primer not given in article – presume mutation from ATG to GTG and GTG to CAG. |

The Ribosome-Binding Site Calculator algorithm includes a consideration of both the ΔG for binding of possible ribosome-binding sites to 16S rRNA and RNA secondary structures that may need to be unfolded for the ribosome to bind. This is why changing the sequence can influence the effectiveness of a distant initiation site. Corrales-Guerrero et al. (2013) interpreted their results as indicating the importance of M1 as the dominant start codon. This may be the case, however, it is also possible, as indicated by the table, that mutating M1 affected the function of the true dominant start codons, M5 and V7.

REFERENCES

Salis, H.M., Mirsky, E.A., Voigt, C.A. (2009). Automated design of synthetic ribosome binding sites to control protein expression. *Nature Biotechnol* **27**: 946-950.

Corrales-Guerrero, L., Mariscal, V., Flores, E., Herrero, A. (2013) Functional dissection and evidence for intercellular transfer of the heterocyst-differentiation PatS morphogen. *Mol Microbiol* **88**: 1093-1105.