Note for cloning of SpyTag-fusion partners: if SpyTag is positioned *right next* to the initiation codon, with certain codon usage we found poor induction in bacteria. This is probably because of secondary structure formation with vector-derived sequences in the mRNA. The codons below worked well for these two common promoter systems:

**T7 vector gcacacatagtaatggtagacgcctacaagccgacgaag**

**T5 vector gctcatatcgtcatggttgacgcgtataaaccgaccaaa**

Ideally you should check your particular construct using an online [Ribosome Binding Site calculator](https://salis.psu.edu/software/) (https://salis.psu.edu/software/), with 10,000 representing a satisfactory score.