***In vitro* characterisation of the MS2 RNA polymerase complex reveals novel host factors modulating leviviral replicase activity**

**Supplementary Information**

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**SUPPLEMENTARY INFORMATION**

# Supplementary Materials & Methods:

***Chemicals and Oligonucleotides:***

All oligos for PCR and cloning, as well as CDSII-T24VN, were purchased from Eurofins Genomics. The template switching oligo TSO-CDSII was purchased from Integrated DNA Technologies (IDT). A 1 mM stock solution of (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl)-1H-imidazol-5(4H)-one (DFHBI-1T, Tocris, 5610) was prepared in dimethylsulfoxide (DMSO, Carl Roth, A994.1). The final 200 µM dilution of DFHBI-1T from this stock was prepared with nuclease-free water.

***PCR & IVT for RNA preparation:***

The templates for IVTs were synthesized by PCR using the primers specified in Supplementary Table 1, the templates shown in *Supplementary Table 2* and Q5 Hot Start High-Fidelity 2x Master Mix (NEB), with final concentrations of 0.02 ng / µL for PCR templates and 1 µM for the corresponding primer mix. Annealing temperatures and elongation times are based on standard PCR recommendations by the manufacturer. After the PCR, IVT templates were purified using Monarch® PCR & DNA Cleanup Kit (NEB). For the IVTs, MEGAshortscript™ T7 Transcription Kit (Thermo Scientific Fisher) was used for F30-Bro templates and MEGAscript™ T7 Transcription Kit (Thermo Scientific Fisher) for MS2 templates, following manufacturer’s recommendations. After TurboDNAse digest at 37°C for 30 minutes, the final IVT products were purified using Monarch® RNA Cleanup Kit (NEB).

***PCR & Assembly for cloning:***

In general, plasmids were cloned using NEBuilder® HiFi DNA Assembly Master Mix to combine the pBAD33 backbone with the gene-specific inserts (*Supplementary Table 3*). Inserts for MS2rep and Qβrep were ordered as gBlocks (IDT). The backbone and other gene-specific inserts were synthesized by PCR with Q5 Hot Start High-Fidelity 2x Master Mix (NEB), using combinations of tag- and gene-specific primers, resulting in an overlap between the different fragments of 30 bp. For HiFi assembly, 100 ng backbone was mixed with one equivalent insert in a 5 µL reaction, incubated at 50°C for 30 minutes and diluted with 15 µL H2O. Subsequently, 2 µL of each reaction were transformed into chemically competent TOP10 *E. coli* cells (Thermo Fisher Scientific) and plated on LB-Lennox agar containing 34 µL / mL chloramphenicol.

***Protein purification:***

For protein purification, small cultures of TOP10 *E. coli* cells harbouring the expression plasmids were grown at 30°C overnight in LB-Lennox containing 34 µg / mL Chloramphenicol. The overnight cultures were used to inoculate 1 L LB-Lennox with Chloramphenicol and these fresh cultures were grown at 30°C to an OD600 of approximately 0.5. Following induction with L-Arabinose (Carl Roth) at a final concentration of 0.2% or 1 mM IPTG, respectively, cultures were grown overnight at 16°C. Cells were harvested by centrifugation at 3200 g and 4°C. For lysis, cell pellets were resuspended in 40 mL / L culture HEPES buffer L (50 mM HEPES·KOH pH 7.5, 250 mM NH4Cl, 10 mM MgCl2, 5 mM DTT, 20 mM imidazole, and 1 mM PMSF) and subsequently lysed by sonication on ice. Cell debris were pelleted by centrifugation at 10,000 g and 4°C for 30 minutes. Before being applied to empty gravity flow columns, the supernatant was incubated at 4°C for 30 minutes with 5 mL / L culture of HisPur™ Ni-NTA resin (Thermo Fisher Scientific) then equilibrated with HEPES buffer W (50 mM HEPES·KOH pH 7.5, 250 mM NH4Cl, 10 mM MgCl2, 5 mM DTT, and 20 mM imidazole). With the exception of MS2rep and Qβrep, washing and elution steps for all proteins were performed as followed: Washing five times with five column volumes HEPES buffer W, then elution three times with three column volumes HEPES buffer E (HEPES buffer W with 300 mM imidazole). For the replicases, the resin was washed twice with five column volumes HEPES buffer W, twice with five column volumes HEPES buffer H (HEPES buffer W with 1 M NH4Cl), and again twice with five column volumes. Elution was performed as described above. Corresponding elution fractions were pooled and concentrated using Merck Millipore Amicon™ Ultra Centrifugal Filter Units (Thermo Fisher Scientific) with a molecular weight cut-off of 10 kDa (3200 g at 4°C). After the initial upconcentration, the buffer was exchanged to HEPES/glycerine buffer (50 mM HEPES·KOH pH 7.5, 100 mM KCl, 10 mM MgCl2, 7 mM DTT, 30 % glycerol) by diluting the protein concentrate in the spin concentrator as much as possible, then concentrating it again as much as possible. This process was repeated three times in total, and final protein preparations were frozen in liquid nitrogen and stored at 80°C.

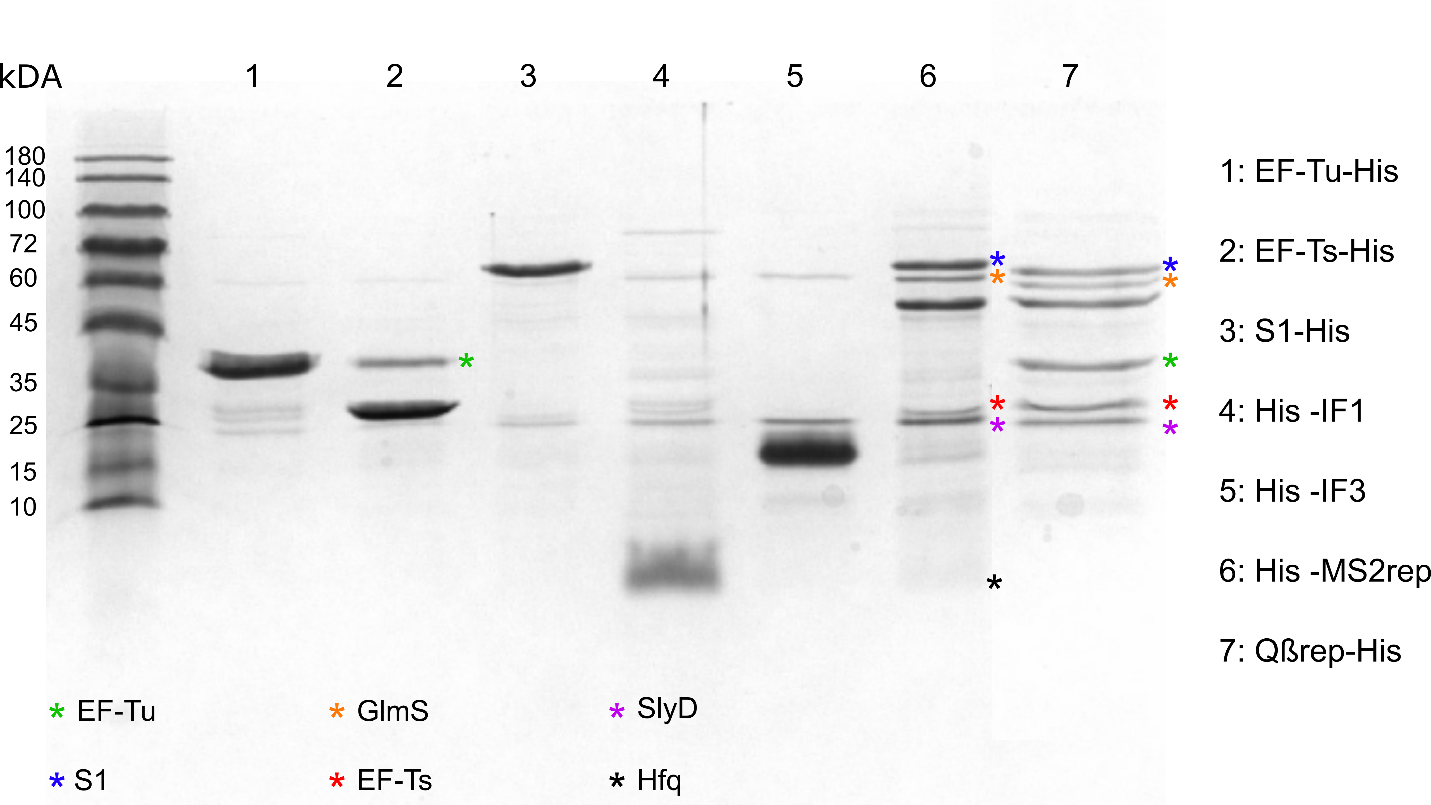
**Supplementary real time fluorescence measurements**

All experiments for supplementary figures were mixed as described in the method section. Subsequently, they were supplemented as followed:

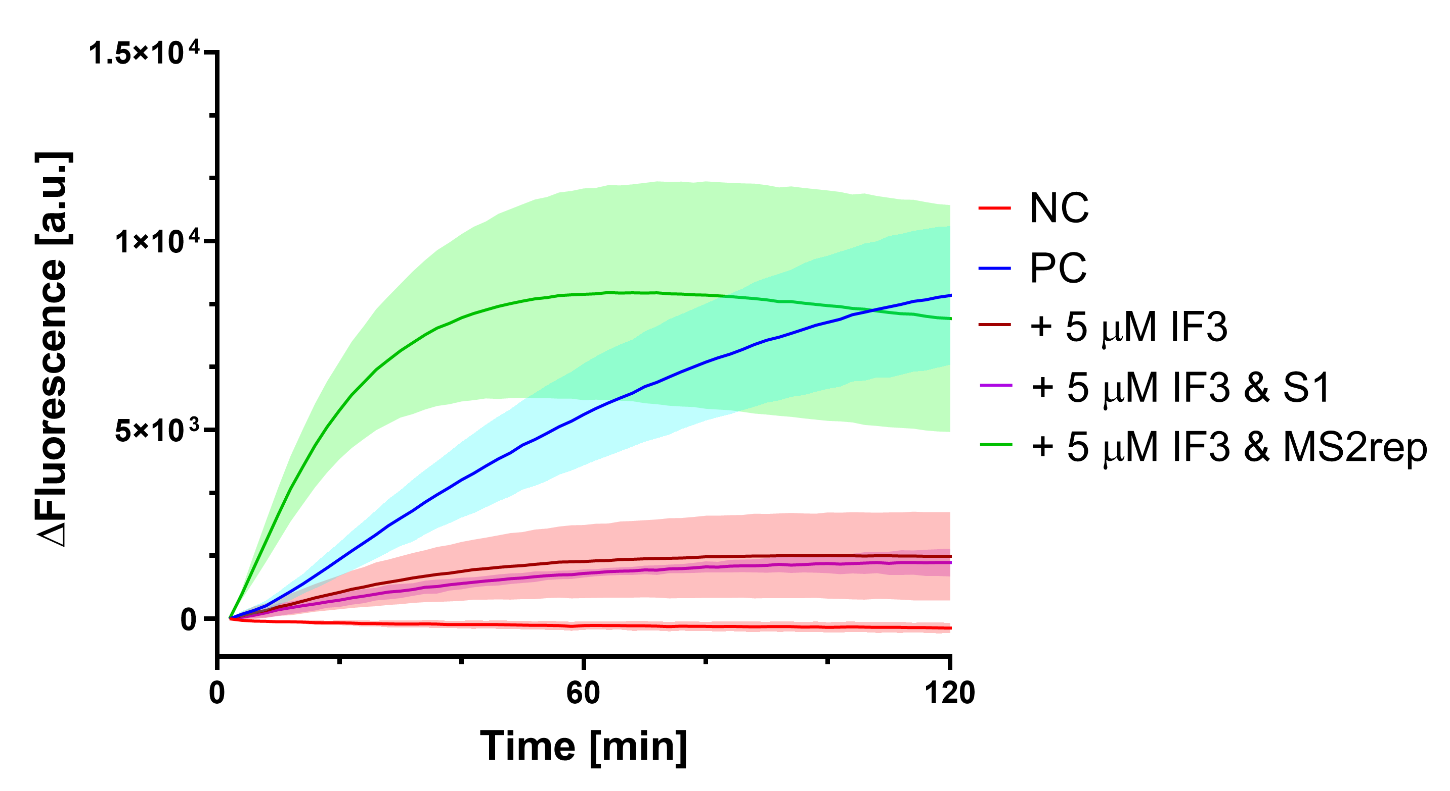
*IF3 inhibition*: 300 nM MS2rep, 50 nM *F30-Bro(-)*, 15 µM EF-Ts, and 10 mM DFHBI-1T

*F30-Bro replication*: 500 nM MS2rep, 25 nM *F30-Bro*, 15 µM EF-Ts, 10 mM DFHBI-1T, and 0.5 U / µL RNase inhibitor (moloX)

# Supplementary Figures:



*Supplementary Figure 1:* Post-purification 17.5% SDS-PAGE of purified cofactors and MS2 or Qβ replicase. 2 µg total protein were loaded per lane. Asterisks indicate co-purified proteins, from top to bottom: EF-Tu (lane 2), S1, GlmS, EF-Ts, SlyD and Hfq (lane 6), S1, GlmS, EF-Tu, EF-Ts, and SlyD (lane 7). For the MS2rep preparation, protein identities of each band were verified by mass spectrometry. SlyD and GlmS are common contaminations during Ni2+ IMAC (1).



Supplementary Figure 2: Influence of IF3 on synthesis of F30-Bro(+). Reactions contained 50 nM F30-Bro(-) template, 15 µM of each EF-Tu and EF-Ts, 1.5 µM S1, 0.3 µM MS2rep (PC), as well the specified combinations of additional protein. In the negative control (NC), MS2rep was omitted. The increase in fluorescence was measured every minute at 37 °C. Error bars indicate standard deviations based on five technical replicates.

Ein Bild, das draußen, Licht, Nacht, dunkel enthält.

Automatisch generierte Beschreibung

Supplementary Figure 3: Gel electrophoresis (1 % agarose) of samples from the replication of full-length MS2 RNA constructs taken after incubation at 37°C for six hours. Core factors (CF) were EF-Ts (15 µM), EF-Tu (15 µM), and S1 (1.5 µM). Red asterisks indicate double-stranded MS2 RNA that migrate at approximately 7200 nt. Purple asterisks indicate small, putatively replicative RNA species. Reactions were prepared as described in the method section using either (+) or (-) strand as templates as indicated on the left side.

Ein Bild, das Licht enthält.

Automatisch generierte Beschreibung

Supplementary Figure 4: Agarose gel electrophoresis (1 % agarose) of a sample taken from an untemplated reaction with purified Qβ replicase as shown in Figure 6A (1 h, 37 °C). Asterisks indicate emerging “RNA parasites”.

# Supplementary Tables

*Supplementary Table 1:* *Primer sequences*

|  |  |  |
| --- | --- | --- |
| **Primer Name** | **Sequence** | **Ta** |
| *PCR for F30-Bro(+), MS2(+)-Bro(-), MS2(+)-Bro(+) & MS2(wt)(+)* | | |
| T7\_MS2(+)aP\_fw | GAAATAATACGACTCACTATAGGGTGGGACCCCTTTC | 67°C |
| MS2\_3endlong\_rev | TGGGTGGTAACTAGCCAAGCAGC |
| *PCR for F30-Bro(-), MS2(-)-Bro(-) & MS2(-)-Bro(+)* | | |
| T7\_MS2(-)aP\_fw | TAATACGACTCACTATAGGGTGGTAAC | 66°C |
| MS2(-)aP\_rv | GGGTGGGACCCCTTTC |
| *RT & Template Switching* | | |
| CDSII-T24VN | AAGCAGTGGTATCAACGCAGAGTTTTTTTTTTTTTTTTTTTTTTTTVN | - |
| TSO-CDSII | GCTAATAAGCAGTGGTATCAACGCAGAGGCAGAGTACATrGrGrG | - |

*Supplementary Table 2: IVT templates*

|  |
| --- |
| **F30-Bro(-)** |
| Broccoli core (-) T7 promoter  TAATACGACTCACTATAGGGTGGTAACTAGCCAAGCAGCTAGTTACCAAATCGGGAGAATCCCGGGTCCTCTCTTTAGGGGGAGGTCCCTGGGCCGAAGCCCGCCCACCTTTCGGTGGAGCCGGACCGCTTTCGCACCCGTGCTCTTTCGAGCACACCCACCCCGTTTACGGGGGTCCCTCGGTCAGCTACCGAGGAGTTGCCATGAATGATCCCGAAGGATCATCAGAGTATGTGGGAGCCCACACTCTACTCGACAGATACGAATATCTGGACCCGACCGTCTCCCACATACACATGGCAAAAACCTCCTAGGAATGGAATTCCGGCTACCTACAGCGATAGCCATGGTAGCGTCTCGCTAAAGACATTAAAAATGGCATTAGCTCGACAGGAAGTTGAGCAGGACCCCGAAAGGGGTCCCACCC |
| **F30-Bro(+)** |
| Broccoli core (+) T7 promoter  TAATACGACTCACTATAGGGTGGGACCCCTTTCGGGGTCCTGCTCAACTTCCTGTCGAGCTAATGCCATTTTTAATGTCTTTAGCGAGACGCTACCATGGCTATCGCTGTAGGTAGCCGGAATTCCATTCCTAGGAGGTTTTTGCCATGTGTATGTGGGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTCCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAACTCCTCGGTAGCTGACCGAGGGACCCCCGTAAACGGGGTGGGTGTGCTCGAAAGAGCACGGGTGCGAAAGCGGTCCGGCTCCACCGAAAGGTGGGCGGGCTTCGGCCCAGGGACCTCCCCCTAAAGAGAGGACCCGGGATTCTCCCGATTTGGTAACTAGCTGCTTGGCTAGTTACCACCC |
| **MS2(+)-Bro(-)** |
| Broccoli core (-) T7 promoter  TAATACGACTCACTATAGGGTGGGACCCCTTTCGGGGTCCTGCTCAACTTCCTGTCGAGCTAATGCCATTTTTAATGTCTTTAGCGAGACGCTACCATGGCTATCGCTGTAGGTAGCCGGAATTCCATTCCTAGGAGGTTTGACCTGTGCGAGCTTTTAGTACCCTTGATAGGGAGAACGAGACCTTCGTCCCCTCCGTTCGCGTTTACGCGGACGGTGAGACTGAAGATAACTCATTCTCTTTAAAATATCGTTCGAACTGGACTCCCGGTCGTTTTAACTCGACTGGGGCCAAAACGAAACAGTGGCACTACCCCTCTCCGTATTCACGGGGGGCGTTAAGTGTCACATCGATAGATCAAGGTGCCTACAAGCGAAGTGGGTCATCGTGGGGTCGCCCGTACGAtGAGAAAGCCGGTTTCGGCTTCTCCCTCGACGCACGCTCCTGCTACAGCCTCTTCCCTGTAAGCCAAAACTTGACTTACATCGAAGTGCCGCAGAACGTTGCGAACCGGGCGTCGACCGAAGTCCTGCAAAAGGTCACCCAGGGTAgTTTTAACCTTGGTGTTGCTcTAGCAGAGGCCAGGTCGACAGCCTCACAACTCGCGACGCAAACCATTGCGCTCGTGAAGGCGTACACTGCCGCTCGTCGCGGTAATTGGCGCCAGGCGCTCCGCTACCTTGCCCTAAACGAAGATCGAAAGTTTCGATCAAAACACGTGGCCGGCAGGTGGTTGGAGTTGCAGTTCGGTTGGTTACCACTAATGAGTGATATCCAGGGTGCATATGAGATGCTTACGAAGGTTCACCTTCAAGAGTTTCTTCCTATGAGAGCCGTACGTCAGGTCGGTACTAACATCAAGTTAGATGGCCGTCTGTCGTATCCAGCTGCAAACTTCCAGACAACGTGCAACATATCGCGACGTATCGTGATATGGTTTTACATAAACGATGCACGTTTGGCATGGTTGTCGTCTCTAGGTATCTTGAACCCACTAGGTATAGTGTGGGAAAAGGTGCCTTTCTCATTCGTTGTCGACTGGCTCCTACCTGTAGGTAACATGCTCGAGGGCCTTACGGCCCCCGTGGGATGCTCCTACATGTCAGGAACAGTTACTGACGTAATAACGGGTGAGTCCATCATAAGCGTTGACGCTCCCTACGGGTGGACTGTGGAGAGACAGGGCACTGCTAAGGCCCAAATCTCAGCCATGCATCGAGGGGTACAATCCGTATGGCCAACAACTGGCGCGTACGTAAAGTCTCCTTTCTCGATGGTCCATACCTTAGATGCGTTAGCATTAATCAGGCAACGGCTCTCTAGATAGAGCCCACACTCTACTCGACAGATACGAATATCTGGACCCGACCGTCTctatctagaggGCCCTCAACCGGAGTTTGAAGCATGGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGACTGTCGCCCCAAGCAACTTCGCTAACGGGGTCGCTGAATGGATCAGCTCTAACTCGCGTTCACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTAAAGTGGCAACCCAGACTGTTGGTGGTGTAGAGCTTCCTGTAGCCGCATGGCGTTCGTACTTAAATATGGAACTAACCATTCCAATTTTCGCTACGAATTCCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCAGCAAACTCCGGCATCTACTAATAGACGCCGGCCATTCAAACATGAGGATTACCCATGTCGAAGACAACAAAGAAGTTCAACTCTTTATGTATTGATCTTCCTCGCGATCTTTCTCTCGAAATTTACCAATCAATTGCTTCTGTCGCTACTGGAAGCGGTGATCCGCACAGTGACGACTTTACAGCAATTGCTTACTTAAGGGACGAATTGCTCACAAAGCATCCGACCTcAGGTTCcGGTAATGACGAGGCGACCCGTCGTACCTTAGCTATCGCTAAGCTACGGGAGGCGAATGaTcggtGCGGTCAGATAAATAGAGAAGGTTTCTTACATGACAAATCCTTGTCATGGGATCCGGATGTTTTACAAACCAGCATCCGTAGCCTTATTGGCAACCTCCTCTCTGGCTACCGATCGTCGTTGTTTGGGCAATGCACGTTCTCCAACGGTGCctCTATGGGGCACAAGTTGCAGGATGCAGCGCCTTACAAGAAGTTCGCTGAACAAGCAACCGTTACCCCCCGCGCTCTGAGAGCGGCTCTATTGGTCCGAGACCAATGTGCGCCGTGGATCAGACACGCGGTCCGCTATAACGAGTCATATGAATTTAGGCTCGTTGTAGGGAACGGAGTGTTTACAGTTCCGAAGAATAATAAAATAGATCGGGCTGCCTGTAAGGAGCCTGATATGAATATGTACCTCCAGAAAGGGGTCGGTGCcTTtATCAGACGCCGGCTCAAATCCGTTGGTATAGACCTaAATGATCAATCGATCAACCAGCGTCTGGCTCAGCAGGGCAGCGTAGATGGTTCGCTTGCGACGATAGACTTATCGTCTGCATCCGATTCCATCTCCGATCGCCTGGTGTGGAGTTTTCTCCCACCtGAGCTATATTCATATCTCGATCGTATCCGCTCACACTACGGAATCGTAGATGGCGAGACGATACGATGGGAACTATTTTCCACAATGGGAAATGGGTTCACATTTGAGCTAGAGTCCATGATATTCTGGGCAATAGTCAAAGCGACCCAAATCCATTTTGGTAACGCCGGAACCATAGGCATCTACGGGGACGATATTATATGTCCCAGTGAGATTGCACCCCGTGTGCTAGAGGCACTTGCCTACTACGGTTTTAAACCGAATCTTCGTAAAACGTTCGTGTCCGGGCTCTTTCGCGAGAGCTGCGGCGCGCACTTTTACCGTGGTGTCGATGTCAAACCGTTTTACATCAAGAAACCTGTTGACAATCTCTTCGCCCTGATGCTGATATTAAATCGGCTACGGGGTTGGGGAGTTGTCGGAGGTATGTCAGATCCACGCCTCTAcAAGGTGTGGGTACGGCTCTCCTCCCAGGTGCCTTCGATGTTCTTCGGTGGGACGGACCTCGCTGCCGACTACTACGTAGTCAGCCCGCCTACGGCAGTCTCGGTATACACCAAGACTCCGcACGGGCGGCTGCTCGCGGATACCCGTACCTCGGGTTTCCGTCTTGCTCGTATCGCTCGAGAACGCAAGTTCTTCAGCGAAAAGCACGACAGTGGTCGCTACATAGCGTGGTTCCATACTGGAGGTGAAATCACCGACAGCATGAAGTCCGCCGGCGTGCGCGTTATACGCACTTCGGAGTGGCTAACGCCGGTTCCCACATTCCCTCAGGAGTGTGGGCCAGCGAGCTCTCCTCGGTAGCTGACCGAGGGACCCCCGTAAACGGGGTGGGTGTGCTCGAAAGAGCACGGGTcCGcgaaaGcggtGGCTCCACCGAAAGGTGGGCGGGCTTCGGCCCAGGGACCTCCCCCTAAAGAGAGGACCCGGGATTCTCCCGATTTGGTAACTAGCTGCTTGGCTAGTTACCACCC |
| **MS2(-)-Bro(-)** |
| Broccoli core (+) T7 promoter  TAATACGACTCACTATAGGGTGGTAACTAGCCAAGCAGCTAGTTACCAAATCGGGAGAATCCCGGGTCCTCTCTTTAGGGGGAGGTCCCTGGGCCGAAGCCCGCCCACCTTTCGGTGGAGCCaccgCtttcgCGgACCCGTGCTCTTTCGAGCACACCCACCCCGTTTACGGGGGTCCCTCGGTCAGCTACCGAGGAGAGCTCGCTGGCCCACACTCCTGAGGGAATGTGGGAACCGGCGTTAGCCACTCCGAAGTGCGTATAACGCGCACGCCGGCGGACTTCATGCTGTCGGTGATTTCACCTCCAGTATGGAACCACGCTATGTAGCGACCACTGTCGTGCTTTTCGCTGAAGAACTTGCGTTCTCGAGCGATACGAGCAAGACGGAAACCCGAGGTACGGGTATCCGCGAGCAGCCGCCCGTgCGGAGTCTTGGTGTATACCGAGACTGCCGTAGGCGGGCTGACTACGTAGTAGTCGGCAGCGAGGTCCGTCCCACCGAAGAACATCGAAGGCACCTGGGAGGAGAGCCGTACCCACACCTTgTAGAGGCGTGGATCTGACATACCTCCGACAACTCCCCAACCCCGTAGCCGATTTAATATCAGCATCAGGGCGAAGAGATTGTCAACAGGTTTCTTGATGTAAAACGGTTTGACATCGACACCACGGTAAAAGTGCGCGCCGCAGCTCTCGCGAAAGAGCCCGGACACGAACGTTTTACGAAGATTCGGTTTAAAACCGTAGTAGGCAAGTGCCTCTAGCACACGGGGTGCAATCTCACTGGGACATATAATATCGTCCCCGTAGATGCCTATGGTTCCGGCGTTACCAAAATGGATTTGGGTCGCTTTGACTATTGCCCAGAATATCATGGACTCTAGCTCAAATGTGAACCCATTTCCCATTGTGGAAAATAGTTCCCATCGTATCGTCTCGCCATCTACGATTCCGTAGTGTGAGCGGATACGATCGAGATATGAATATAGCTCaGGTGGGAGAAAACTCCACACCAGGCGATCGGAGATGGAATCGGATGCAGACGATAAGTCTATCGTCGCAAGCGAACCATCTACGCTGCCCTGCTGAGCCAGACGCTGGTTGATCGATTGATCATTtAGGTCTATACCAACGGATTTGAGCCGGCGTCTGATaAAgGCACCGACCCCTTTCTGGAGGTACATATTCATATCAGGCTCCTTACAGGCAGCCCGATCTATTTTATTATTCTTCGGAACTGTAAACACTCCGTTCCCTACAACGAGCCTAAATTCATATGACTCGTTATAGCGGACCGCGTGTCTGATCCACGGCGCACATTGGTCTCGGACCAATAGAGCCGCTCTCAGAGCGCGGGGGGTAACGGTTGCTTGTTCAGCGAACTTCTTGTAAGGCGCTGCATCCTGCAACTTGTGCCCCATAGagGCACCGTTGGAGAACGTGCATTGCCCAAACAACGACGATCGGTAGCCAGAGAGGAGGTTGCCAATAAGGCTACGGATGCTGGTTTGTAAAACATCCGGATCCCATGACAAGGATTTGTCATGTAAGAAACCTTCTCTATTTATCTGACCGCaccgAtCATTCGCCTCCCGTAGCTTAGCGATAGCTAAGGTACGACGGGTCGCCTCGTCATTACCgGAACCTgAGGTCGGATGCTTTGTGAGCAATTCGTCCCTTAAGTAAGCAATTGCTGTAAAGTCGTCACTGTGCGGATCACCGCTTCCAGTAGCGACAGAAGCAATTGATTGGTAAATTTCGAGAGAAAGATCGCGAGGAAGATCAATACATAAAGAGTTGAACTTCTTTGTTGTCTTCGACATGGGTAATCCTCATGTTTGAATGGCCGGCGTCTATTAGTAGATGCCGGAGTTTGCTGCGATTGCTGAGGGAATCGGGTTTCCATCTTTTAGGAGACCTTGCATTGCCTTAACAATAAGCTCGCAGTCGGAATTCGTAGCGAAAATTGGAATGGTTAGTTCCATATTTAAGTACGAACGCCATGCGGCTACAGGAAGCTCTACACCACCAACAGTCTGGGTTGCCACTTTAGGCACCTCGACTTTGATGGTGTATTTGCGATTCTGCGCAGAGCTCTGACGAACGCTACAGGTTACTTTGTAAGCCTGTGAACGCGAGTTAGAGCTGATCCATTCAGCGACCCCGTTAGCGAAGTTGCTTGGGGCGACAGTCACGTCGCCAGTTCCGCCATTGTCGACGAGAACGAACTGAGTAAAGTTAGAAGCCATGCTTCAAACTCCGGTTGAGGGCcctctagatagAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTCTATCTAGAGAGCCGTTGCCTGATTAATGCTAACGCATCTAAGGTATGGACCATCGAGAAAGGAGACTTTACGTACGCGCCAGTTGTTGGCCATACGGATTGTACCCCTCGATGCATGGCTGAGATTTGGGCCTTAGCAGTGCCCTGTCTCTCCACAGTCCACCCGTAGGGAGCGTCAACGCTTATGATGGACTCACCCGTTATTACGTCAGTAACTGTTCCTGACATGTAGGAGCATCCCACGGGGGCCGTAAGGCCCTCGAGCATGTTACCTACAGGTAGGAGCCAGTCGACAACGAATGAGAAAGGCACCTTTTCCCACACTATACCTAGTGGGTTCAAGATACCTAGAGACGACAACCATGCCAAACGTGCATCGTTTATGTAAAACCATATCACGATACGTCGCGATATGTTGCACGTTGTCTGGAAGTTTGCAGCTGGATACGACAGACGGCCATCTAACTTGATGTTAGTACCGACCTGACGTACGGCTCTCATAGGAAGAAACTCTTGAAGGTGAACCTTCGTAAGCATCTCATATGCACCCTGGATATCACTCATTAGTGGTAACCAACCGAACTGCAACTCCAACCACCTGCCGGCCACGTGTTTTGATCGAAACTTTCGATCTTCGTTTAGGGCAAGGTAGCGGAGCGCCTGGCGCCAATTACCGCGACGAGCGGCAGTGTACGCCTTCACGAGCGCAATGGTTTGCGTCGCGAGTTGTGAGGCTGTCGACCTGGCCTCTGCTAgAGCAACACCAAGGTTAAAAcTACCCTGGGTGACCTTTTGCAGGACTTCGGTCGACGCCCGGTTCGCAACGTTCTGCGGCACTTCGATGTAAGTCAAGTTTTGGCTTACAGGGAAGAGGCTGTAGCAGGAGCGTGCGTCGAGGGAGAAGCCGAAACCGGCTTTCTCaTCGTACGGGCGACCCCACGATGACCCACTTCGCTTGTAGGCACCTTGATCTATCGATGTGACACTTAACGCCCCCCGTGAATACGGAGAGGGGTAGTGCCACTGTTTCGTTTTGGCCCCAGTCGAGTTAAAACGACCGGGAGTCCAGTTCGAACGATATTTTAAAGAGAATGAGTTATCTTCAGTCTCACCGTCCGCGTAAACGCGAACGGAGGGGACGAAGGTCTCGTTCTCCCTATCAAGGGTACTAAAAGCTCGCACAGGTCAAACCTCCTAGGAATGGAATTCCGGCTACCTACAGCGATAGCCATGGTAGCGTCTCGCTAAAGACATTAAAAATGGCATTAGCTCGACAGGAAGTTGAGCAGGACCCCGAAAGGGGTCCCACCC |
| **MS2(+)-Bro(+)** |
| Broccoli core (+) T7 promoter  TAATACGACTCACTATAGGGTGGGACCCCTTTCGGGGTCCTGCTCAACTTCCTGTCGAGCTAATGCCATTTTTAATGTCTTTAGCGAGACGCTACCATGGCTATCGCTGTAGGTAGCCGGAATTCCATTCCTAGGAGGTTTGACCTGTGCGAGCTTTTAGTACCCTTGATAGGGAGAACGAGACCTTCGTCCCCTCCGTTCGCGTTTACGCGGACGGTGAGACTGAAGATAACTCATTCTCTTTAAAATATCGTTCGAACTGGACTCCCGGTCGTTTTAACTCGACTGGGGCCAAAACGAAACAGTGGCACTACCCCTCTCCGTATTCACGGGGGGCGTTAAGTGTCACATCGATAGATCAAGGTGCCTACAAGCGAAGTGGGTCATCGTGGGGTCGCCCGTACGAtGAGAAAGCCGGTTTCGGCTTCTCCCTCGACGCACGCTCCTGCTACAGCCTCTTCCCTGTAAGCCAAAACTTGACTTACATCGAAGTGCCGCAGAACGTTGCGAACCGGGCGTCGACCGAAGTCCTGCAAAAGGTCACCCAGGGTAgTTTTAACCTTGGTGTTGCTcTAGCAGAGGCCAGGTCGACAGCCTCACAACTCGCGACGCAAACCATTGCGCTCGTGAAGGCGTACACTGCCGCTCGTCGCGGTAATTGGCGCCAGGCGCTCCGCTACCTTGCCCTAAACGAAGATCGAAAGTTTCGATCAAAACACGTGGCCGGCAGGTGGTTGGAGTTGCAGTTCGGTTGGTTACCACTAATGAGTGATATCCAGGGTGCATATGAGATGCTTACGAAGGTTCACCTTCAAGAGTTTCTTCCTATGAGAGCCGTACGTCAGGTCGGTACTAACATCAAGTTAGATGGCCGTCTGTCGTATCCAGCTGCAAACTTCCAGACAACGTGCAACATATCGCGACGTATCGTGATATGGTTTTACATAAACGATGCACGTTTGGCATGGTTGTCGTCTCTAGGTATCTTGAACCCACTAGGTATAGTGTGGGAAAAGGTGCCTTTCTCATTCGTTGTCGACTGGCTCCTACCTGTAGGTAACATGCTCGAGGGCCTTACGGCCCCCGTGGGATGCTCCTACATGTCAGGAACAGTTACTGACGTAATAACGGGTGAGTCCATCATAAGCGTTGACGCTCCCTACGGGTGGACTGTGGAGAGACAGGGCACTGCTAAGGCCCAAATCTCAGCCATGCATCGAGGGGTACAATCCGTATGGCCAACAACTGGCGCGTACGTAAAGTCTCCTTTCTCGATGGTCCATACCTTAGATGCGTTAGCATTAATCAGGCAACGGCTCTCTAGATAGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTctatctagaggGCCCTCAACCGGAGTTTGAAGCATGGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGACTGTCGCCCCAAGCAACTTCGCTAACGGGGTCGCTGAATGGATCAGCTCTAACTCGCGTTCACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTAAAGTGGCAACCCAGACTGTTGGTGGTGTAGAGCTTCCTGTAGCCGCATGGCGTTCGTACTTAAATATGGAACTAACCATTCCAATTTTCGCTACGAATTCCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCAGCAAACTCCGGCATCTACTAATAGACGCCGGCCATTCAAACATGAGGATTACCCATGTCGAAGACAACAAAGAAGTTCAACTCTTTATGTATTGATCTTCCTCGCGATCTTTCTCTCGAAATTTACCAATCAATTGCTTCTGTCGCTACTGGAAGCGGTGATCCGCACAGTGACGACTTTACAGCAATTGCTTACTTAAGGGACGAATTGCTCACAAAGCATCCGACCTcAGGTTCcGGTAATGACGAGGCGACCCGTCGTACCTTAGCTATCGCTAAGCTACGGGAGGCGAATGaTcggtGCGGTCAGATAAATAGAGAAGGTTTCTTACATGACAAATCCTTGTCATGGGATCCGGATGTTTTACAAACCAGCATCCGTAGCCTTATTGGCAACCTCCTCTCTGGCTACCGATCGTCGTTGTTTGGGCAATGCACGTTCTCCAACGGTGCctCTATGGGGCACAAGTTGCAGGATGCAGCGCCTTACAAGAAGTTCGCTGAACAAGCAACCGTTACCCCCCGCGCTCTGAGAGCGGCTCTATTGGTCCGAGACCAATGTGCGCCGTGGATCAGACACGCGGTCCGCTATAACGAGTCATATGAATTTAGGCTCGTTGTAGGGAACGGAGTGTTTACAGTTCCGAAGAATAATAAAATAGATCGGGCTGCCTGTAAGGAGCCTGATATGAATATGTACCTCCAGAAAGGGGTCGGTGCcTTtATCAGACGCCGGCTCAAATCCGTTGGTATAGACCTaAATGATCAATCGATCAACCAGCGTCTGGCTCAGCAGGGCAGCGTAGATGGTTCGCTTGCGACGATAGACTTATCGTCTGCATCCGATTCCATCTCCGATCGCCTGGTGTGGAGTTTTCTCCCACCtGAGCTATATTCATATCTCGATCGTATCCGCTCACACTACGGAATCGTAGATGGCGAGACGATACGATGGGAACTATTTTCCACAATGGGAAATGGGTTCACATTTGAGCTAGAGTCCATGATATTCTGGGCAATAGTCAAAGCGACCCAAATCCATTTTGGTAACGCCGGAACCATAGGCATCTACGGGGACGATATTATATGTCCCAGTGAGATTGCACCCCGTGTGCTAGAGGCACTTGCCTACTACGGTTTTAAACCGAATCTTCGTAAAACGTTCGTGTCCGGGCTCTTTCGCGAGAGCTGCGGCGCGCACTTTTACCGTGGTGTCGATGTCAAACCGTTTTACATCAAGAAACCTGTTGACAATCTCTTCGCCCTGATGCTGATATTAAATCGGCTACGGGGTTGGGGAGTTGTCGGAGGTATGTCAGATCCACGCCTCTAcAAGGTGTGGGTACGGCTCTCCTCCCAGGTGCCTTCGATGTTCTTCGGTGGGACGGACCTCGCTGCCGACTACTACGTAGTCAGCCCGCCTACGGCAGTCTCGGTATACACCAAGACTCCGcACGGGCGGCTGCTCGCGGATACCCGTACCTCGGGTTTCCGTCTTGCTCGTATCGCTCGAGAACGCAAGTTCTTCAGCGAAAAGCACGACAGTGGTCGCTACATAGCGTGGTTCCATACTGGAGGTGAAATCACCGACAGCATGAAGTCCGCCGGCGTGCGCGTTATACGCACTTCGGAGTGGCTAACGCCGGTTCCCACATTCCCTCAGGAGTGTGGGCCAGCGAGCTCTCCTCGGTAGCTGACCGAGGGACCCCCGTAAACGGGGTGGGTGTGCTCGAAAGAGCACGGGTcCGcgaaaGcggtGGCTCCACCGAAAGGTGGGCGGGCTTCGGCCCAGGGACCTCCCCCTAAAGAGAGGACCCGGGATTCTCCCGATTTGGTAACTAGCTGCTTGGCTAGTTACCACCC |
| **MS2(-)-Bro(+)** |
| Broccoli core (-) T7 promoter  TAATACGACTCACTATAGGGTGGTAACTAGCCAAGCAGCTAGTTACCAAATCGGGAGAATCCCGGGTCCTCTCTTTAGGGGGAGGTCCCTGGGCCGAAGCCCGCCCACCTTTCGGTGGAGCCaccgCtttcgCGgACCCGTGCTCTTTCGAGCACACCCACCCCGTTTACGGGGGTCCCTCGGTCAGCTACCGAGGAGAGCTCGCTGGCCCACACTCCTGAGGGAATGTGGGAACCGGCGTTAGCCACTCCGAAGTGCGTATAACGCGCACGCCGGCGGACTTCATGCTGTCGGTGATTTCACCTCCAGTATGGAACCACGCTATGTAGCGACCACTGTCGTGCTTTTCGCTGAAGAACTTGCGTTCTCGAGCGATACGAGCAAGACGGAAACCCGAGGTACGGGTATCCGCGAGCAGCCGCCCGTgCGGAGTCTTGGTGTATACCGAGACTGCCGTAGGCGGGCTGACTACGTAGTAGTCGGCAGCGAGGTCCGTCCCACCGAAGAACATCGAAGGCACCTGGGAGGAGAGCCGTACCCACACCTTgTAGAGGCGTGGATCTGACATACCTCCGACAACTCCCCAACCCCGTAGCCGATTTAATATCAGCATCAGGGCGAAGAGATTGTCAACAGGTTTCTTGATGTAAAACGGTTTGACATCGACACCACGGTAAAAGTGCGCGCCGCAGCTCTCGCGAAAGAGCCCGGACACGAACGTTTTACGAAGATTCGGTTTAAAACCGTAGTAGGCAAGTGCCTCTAGCACACGGGGTGCAATCTCACTGGGACATATAATATCGTCCCCGTAGATGCCTATGGTTCCGGCGTTACCAAAATGGATTTGGGTCGCTTTGACTATTGCCCAGAATATCATGGACTCTAGCTCAAATGTGAACCCATTTCCCATTGTGGAAAATAGTTCCCATCGTATCGTCTCGCCATCTACGATTCCGTAGTGTGAGCGGATACGATCGAGATATGAATATAGCTCaGGTGGGAGAAAACTCCACACCAGGCGATCGGAGATGGAATCGGATGCAGACGATAAGTCTATCGTCGCAAGCGAACCATCTACGCTGCCCTGCTGAGCCAGACGCTGGTTGATCGATTGATCATTtAGGTCTATACCAACGGATTTGAGCCGGCGTCTGATaAAgGCACCGACCCCTTTCTGGAGGTACATATTCATATCAGGCTCCTTACAGGCAGCCCGATCTATTTTATTATTCTTCGGAACTGTAAACACTCCGTTCCCTACAACGAGCCTAAATTCATATGACTCGTTATAGCGGACCGCGTGTCTGATCCACGGCGCACATTGGTCTCGGACCAATAGAGCCGCTCTCAGAGCGCGGGGGGTAACGGTTGCTTGTTCAGCGAACTTCTTGTAAGGCGCTGCATCCTGCAACTTGTGCCCCATAGagGCACCGTTGGAGAACGTGCATTGCCCAAACAACGACGATCGGTAGCCAGAGAGGAGGTTGCCAATAAGGCTACGGATGCTGGTTTGTAAAACATCCGGATCCCATGACAAGGATTTGTCATGTAAGAAACCTTCTCTATTTATCTGACCGCaccgAtCATTCGCCTCCCGTAGCTTAGCGATAGCTAAGGTACGACGGGTCGCCTCGTCATTACCgGAACCTgAGGTCGGATGCTTTGTGAGCAATTCGTCCCTTAAGTAAGCAATTGCTGTAAAGTCGTCACTGTGCGGATCACCGCTTCCAGTAGCGACAGAAGCAATTGATTGGTAAATTTCGAGAGAAAGATCGCGAGGAAGATCAATACATAAAGAGTTGAACTTCTTTGTTGTCTTCGACATGGGTAATCCTCATGTTTGAATGGCCGGCGTCTATTAGTAGATGCCGGAGTTTGCTGCGATTGCTGAGGGAATCGGGTTTCCATCTTTTAGGAGACCTTGCATTGCCTTAACAATAAGCTCGCAGTCGGAATTCGTAGCGAAAATTGGAATGGTTAGTTCCATATTTAAGTACGAACGCCATGCGGCTACAGGAAGCTCTACACCACCAACAGTCTGGGTTGCCACTTTAGGCACCTCGACTTTGATGGTGTATTTGCGATTCTGCGCAGAGCTCTGACGAACGCTACAGGTTACTTTGTAAGCCTGTGAACGCGAGTTAGAGCTGATCCATTCAGCGACCCCGTTAGCGAAGTTGCTTGGGGCGACAGTCACGTCGCCAGTTCCGCCATTGTCGACGAGAACGAACTGAGTAAAGTTAGAAGCCATGCTTCAAACTCCGGTTGAGGGCcctctagatagAGCCCACACTCTACTCGACAGATACGAATATCTGGACCCGACCGTCTCTATCTAGAGAGCCGTTGCCTGATTAATGCTAACGCATCTAAGGTATGGACCATCGAGAAAGGAGACTTTACGTACGCGCCAGTTGTTGGCCATACGGATTGTACCCCTCGATGCATGGCTGAGATTTGGGCCTTAGCAGTGCCCTGTCTCTCCACAGTCCACCCGTAGGGAGCGTCAACGCTTATGATGGACTCACCCGTTATTACGTCAGTAACTGTTCCTGACATGTAGGAGCATCCCACGGGGGCCGTAAGGCCCTCGAGCATGTTACCTACAGGTAGGAGCCAGTCGACAACGAATGAGAAAGGCACCTTTTCCCACACTATACCTAGTGGGTTCAAGATACCTAGAGACGACAACCATGCCAAACGTGCATCGTTTATGTAAAACCATATCACGATACGTCGCGATATGTTGCACGTTGTCTGGAAGTTTGCAGCTGGATACGACAGACGGCCATCTAACTTGATGTTAGTACCGACCTGACGTACGGCTCTCATAGGAAGAAACTCTTGAAGGTGAACCTTCGTAAGCATCTCATATGCACCCTGGATATCACTCATTAGTGGTAACCAACCGAACTGCAACTCCAACCACCTGCCGGCCACGTGTTTTGATCGAAACTTTCGATCTTCGTTTAGGGCAAGGTAGCGGAGCGCCTGGCGCCAATTACCGCGACGAGCGGCAGTGTACGCCTTCACGAGCGCAATGGTTTGCGTCGCGAGTTGTGAGGCTGTCGACCTGGCCTCTGCTAgAGCAACACCAAGGTTAAAAcTACCCTGGGTGACCTTTTGCAGGACTTCGGTCGACGCCCGGTTCGCAACGTTCTGCGGCACTTCGATGTAAGTCAAGTTTTGGCTTACAGGGAAGAGGCTGTAGCAGGAGCGTGCGTCGAGGGAGAAGCCGAAACCGGCTTTCTCaTCGTACGGGCGACCCCACGATGACCCACTTCGCTTGTAGGCACCTTGATCTATCGATGTGACACTTAACGCCCCCCGTGAATACGGAGAGGGGTAGTGCCACTGTTTCGTTTTGGCCCCAGTCGAGTTAAAACGACCGGGAGTCCAGTTCGAACGATATTTTAAAGAGAATGAGTTATCTTCAGTCTCACCGTCCGCGTAAACGCGAACGGAGGGGACGAAGGTCTCGTTCTCCCTATCAAGGGTACTAAAAGCTCGCACAGGTCAAACCTCCTAGGAATGGAATTCCGGCTACCTACAGCGATAGCCATGGTAGCGTCTCGCTAAAGACATTAAAAATGGCATTAGCTCGACAGGAAGTTGAGCAGGACCCCGAAAGGGGTCCCACCC |
| **MS2(wt)(+)** |
| T7 promoter  TAATACGACTCACTATAGGGTGGGACCCCTTTCGGGGTCCTGCTCAACTTCCTGTCGAGCTAATGCCATTTTTAATGTCTTTAGCGAGACGCTACCATGGCTATCGCTGTAGGTAGCCGGAATTCCATTCCTAGGAGGTTTGACCTGTGCGAGCTTTTAGTACCCTTGATAGGGAGAACGAGACCTTCGTCCCCTCCGTTCGCGTTTACGCGGACGGTGAGACTGAAGATAACTCATTCTCTTTAAAATATCGTTCGAACTGGACTCCCGGTCGTTTTAACTCGACTGGGGCCAAAACGAAACAGTGGCACTACCCCTCTCCGTATTCACGGGGGGCGTTAAGTGTCACATCGATAGATCAAGGTGCCTACAAGCGAAGTGGGTCATCGTGGGGTCGCCCGTACGAtGAGAAAGCCGGTTTCGGCTTCTCCCTCGACGCACGCTCCTGCTACAGCCTCTTCCCTGTAAGCCAAAACTTGACTTACATCGAAGTGCCGCAGAACGTTGCGAACCGGGCGTCGACCGAAGTCCTGCAAAAGGTCACCCAGGGTAgTTTTAACCTTGGTGTTGCTcTAGCAGAGGCCAGGTCGACAGCCTCACAACTCGCGACGCAAACCATTGCGCTCGTGAAGGCGTACACTGCCGCTCGTCGCGGTAATTGGCGCCAGGCGCTCCGCTACCTTGCCCTAAACGAAGATCGAAAGTTTCGATCAAAACACGTGGCCGGCAGGTGGTTGGAGTTGCAGTTCGGTTGGTTACCACTAATGAGTGATATCCAGGGTGCATATGAGATGCTTACGAAGGTTCACCTTCAAGAGTTTCTTCCTATGAGAGCCGTACGTCAGGTCGGTACTAACATCAAGTTAGATGGCCGTCTGTCGTATCCAGCTGCAAACTTCCAGACAACGTGCAACATATCGCGACGTATCGTGATATGGTTTTACATAAACGATGCACGTTTGGCATGGTTGTCGTCTCTAGGTATCTTGAACCCACTAGGTATAGTGTGGGAAAAGGTGCCTTTCTCATTCGTTGTCGACTGGCTCCTACCTGTAGGTAACATGCTCGAGGGCCTTACGGCCCCCGTGGGATGCTCCTACATGTCAGGAACAGTTACTGACGTAATAACGGGTGAGTCCATCATAAGCGTTGACGCTCCCTACGGGTGGACTGTGGAGAGACAGGGCACTGCTAAGGCCCAAATCTCAGCCATGCATCGAGGGGTACAATCCGTATGGCCAACAACTGGCGCGTACGTAAAGTCTCCTTTCTCGATGGTCCATACCTTAGATGCGTTAGCATTAATCAGGCAACGGCTCTCTAGATAGgGCCCTCAACCGGAGTTTGAAGCATGGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGACTGTCGCCCCAAGCAACTTCGCTAACGGGGTCGCTGAATGGATCAGCTCTAACTCGCGTTCACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTAAAGTGGCAACCCAGACTGTTGGTGGTGTAGAGCTTCCTGTAGCCGCATGGCGTTCGTACTTAAATATGGAACTAACCATTCCAATTTTCGCTACGAATTCCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCAGCAAACTCCGGCATCTACTAATAGACGCCGGCCATTCAAACATGAGGATTACCCATGTCGAAGACAACAAAGAAGTTCAACTCTTTATGTATTGATCTTCCTCGCGATCTTTCTCTCGAAATTTACCAATCAATTGCTTCTGTCGCTACTGGAAGCGGTGATCCGCACAGTGACGACTTTACAGCAATTGCTTACTTAAGGGACGAATTGCTCACAAAGCATCCGACCTcAGGTTCcGGTAATGACGAGGCGACCCGTCGTACCTTAGCTATCGCTAAGCTACGGGAGGCGAATGaTcggtGCGGTCAGATAAATAGAGAAGGTTTCTTACATGACAAATCCTTGTCATGGGATCCGGATGTTTTACAAACCAGCATCCGTAGCCTTATTGGCAACCTCCTCTCTGGCTACCGATCGTCGTTGTTTGGGCAATGCACGTTCTCCAACGGTGCctCTATGGGGCACAAGTTGCAGGATGCAGCGCCTTACAAGAAGTTCGCTGAACAAGCAACCGTTACCCCCCGCGCTCTGAGAGCGGCTCTATTGGTCCGAGACCAATGTGCGCCGTGGATCAGACACGCGGTCCGCTATAACGAGTCATATGAATTTAGGCTCGTTGTAGGGAACGGAGTGTTTACAGTTCCGAAGAATAATAAAATAGATCGGGCTGCCTGTAAGGAGCCTGATATGAATATGTACCTCCAGAAAGGGGTCGGTGCcTTtATCAGACGCCGGCTCAAATCCGTTGGTATAGACCTaAATGATCAATCGATCAACCAGCGTCTGGCTCAGCAGGGCAGCGTAGATGGTTCGCTTGCGACGATAGACTTATCGTCTGCATCCGATTCCATCTCCGATCGCCTGGTGTGGAGTTTTCTCCCACCtGAGCTATATTCATATCTCGATCGTATCCGCTCACACTACGGAATCGTAGATGGCGAGACGATACGATGGGAACTATTTTCCACAATGGGAAATGGGTTCACATTTGAGCTAGAGTCCATGATATTCTGGGCAATAGTCAAAGCGACCCAAATCCATTTTGGTAACGCCGGAACCATAGGCATCTACGGGGACGATATTATATGTCCCAGTGAGATTGCACCCCGTGTGCTAGAGGCACTTGCCTACTACGGTTTTAAACCGAATCTTCGTAAAACGTTCGTGTCCGGGCTCTTTCGCGAGAGCTGCGGCGCGCACTTTTACCGTGGTGTCGATGTCAAACCGTTTTACATCAAGAAACCTGTTGACAATCTCTTCGCCCTGATGCTGATATTAAATCGGCTACGGGGTTGGGGAGTTGTCGGAGGTATGTCAGATCCACGCCTCTAcAAGGTGTGGGTACGGCTCTCCTCCCAGGTGCCTTCGATGTTCTTCGGTGGGACGGACCTCGCTGCCGACTACTACGTAGTCAGCCCGCCTACGGCAGTCTCGGTATACACCAAGACTCCGcACGGGCGGCTGCTCGCGGATACCCGTACCTCGGGTTTCCGTCTTGCTCGTATCGCTCGAGAACGCAAGTTCTTCAGCGAAAAGCACGACAGTGGTCGCTACATAGCGTGGTTCCATACTGGAGGTGAAATCACCGACAGCATGAAGTCCGCCGGCGTGCGCGTTATACGCACTTCGGAGTGGCTAACGCCGGTTCCCACATTCCCTCAGGAGTGTGGGCCAGCGAGCTCTCCTCGGTAGCTGACCGAGGGACCCCCGTAAACGGGGTGGGTGTGCTCGAAAGAGCACGGGTcCGcgaaaGcggtGGCTCCACCGAAAGGTGGGCGGGCTTCGGCCCAGGGACCTCCCCCTAAAGAGAGGACCCGGGATTCTCCCGATTTGGTAACTAGCTGCTTGGCTAGTTACCACCC |
| **MSRP-22 anti** |
| T7 promoter  TAATACGACTCACTATAAGGGGTGGTAACTAGCCAATCAGCTAGTTACCAAACCGGTAGAATCCCGGGTCCTCTCTTTAGGGGGAGGTCCCTGGGCCGAAGCCCCGCCCACCTTTCGGTGGAGCCCTAGGAATGGAATTCCGGCTACCTACAGCGATAGCCATGGTAGCGTCTCGCTAAAGACATTAAAAATGGCATTAGCTCGACAGGAAGTTGAGCAGGACCCCGAAAGGGGTCCCACCCA |
| **RQ-135-** |
| T7 promoter  TAATACGACTCACTATAGGGGTTCCAACCGGAAGTTGAGGGATGCCTAGGCATCCCCCGTGCGTCCCTTCGATCCTACGAGGGATTTGAGAGATGCCTAGGCATCTCCCGCGCGCCGGTTTCGGACCTCCAGTGCGTGTTACCGCACTGTTAGCCC |

*Supplementary Table 3: Expression plasmids*

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| **pBAD33 backbone** |
| P15A ori CmR araBp  TTTTGTATAGAATTTACGAAGCTTGGCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCAGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTTGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTCAGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTCAATAAACCGGTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCTGCCCTGAACCGACGACCGGGTCGAATTTGCTTTCGAATTTCTGCCATTCATCCGCTTATTATCACTTATTCAGGCGTAGCACCAGGCGTTTAAGGGCACCAATAACTGCCTTAAAAAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAGAAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGGAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCCGTCACAGGTATTTATTCGGCGCAAAGTGCGTCGGGTGATGCTGCCAACTTACTGATTTAGTGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGCTGTCCCTCCTGTTCAGCTACTGACGGGGTGGTGCGTAACGGCAAAAGCACCGCCGGACATCAGCGCTAGCGGAGTGTATACTGGCTTACTATGTTGGCACTGATGAGGGTGTCAGTGAAGTGCTTCATGTGGCAGGAGAAAAAAGGCTGCACCGGTGCGTCAGCAGAATATGTGATACAGGATATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCGTTCGACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATACTTAACAGGGAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATCACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCGGTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGGTAGGCAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACCACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTTCGTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATCAGATAAAATATTTCTAGGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCTGCTCATGTTTGACAGCTTATCATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGGATTCTGCAAACCCTATGCTACTCCGTCAAGCCGTCAATTGTCTGATTCGTTACCAATTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGACGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTAC CCGATTATCCATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCAGATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTCATGCCAGTAGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTCGCGAGCCTCCGGATGACGACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAACAAATTCTCGTCCCTGATTTTTCACCACCCCCTGACCGCGAATGGTGAGATTGAGAATATAACCTTTCATTCCCAGCGGTCGGTCGATAAAAAAATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCATTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTTTCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGAATTCACC |
| **His6-MS2rep insert** |
| Start Stop  ATGCATCACCATCACCATCACTCGAAGACAACAAAGAAGTTCAACTCTTTATGTATTGATCTTCCTCGCGATCTTTCTCTCGAAATTTACCAATCAATTGCTTCTGTCGCTACTGGAAGCGGTGATCCGCACAGTGACGACTTTACAGCAATTGCTTACTTAAGGGACGAATTGCTCACAAAGCATCCGACCTTAGGTTCTGGTAATGACGAGGCGACCCGTCGTACCTTAGCTATCGCTAAGCTACGGGAGGCGAATGATCGGTGCGGTCAGATAAATAGAGAAGGTTTCTTACATGACAAATCCTTGTCATGGGATCCGGATGTTTTACAAACCAGCATCCGTAGCCTTATTGGCAACCTCCTCTCTGGCTACCGATCGTCGTTGTTTGGGCAATGCACGTTCTCCAACGGTGCCTCTATGGGGCACAAGTTGCAGGATGCAGCGCCCTACAAGAAGTTCGCTGAACAAGCAACCGTTACCCCCCGCGCTCTGAGAGCGGCTCTATTGGTCCGAGACCAATGTGCGCCGTGGATCAGACACGCGGTCCGCTATAACGAGTCATATGAGTTTAGGCTCGTTGTAGGGAACGGAGTGTTTACAGTTCCGAAGAATAATAAAATAGATCGGGCTGCCTGTAAGGAGCCTGATATGAATATGTACCTCCAGAAAGGGGTCGGTGCCTTTATCAGACGCCGGCTCAAATCCGTTGGTATAGACCTGAATGATCAATCGATCAACCAGCGTCTGGCTCAGCAGGGCAGCGTAGATGGTTCGCTTGCGACGATAGACTTATCGTCTGCATCCGATTCCATCTCCGATCGCCTGGTGTGGAGTTTTCTCCCACCTGAGCTATATTCATATCTCGATCGTATCCGCTCACACTACGGAATCGTAGATGGCGAGACGATACGATGGGAACTATTTTCCACAATGGGAAATGGGTTCACATTTGAGCTAGAGTCCATGATATTCTGGGCAATAGTCAAAGCGACCCAAATCCATTTTGGTAACGCCGGAACCATAGGCATCTACGGGGACGATATTATATGCCCCAGTGAGATTGCACCCCGTGTGCTAGAGGCACTTGCCTACTACGGTTTTAAACCGAATCTTCGCAAAACGTTCGTGTCCGGGCTCTTTCGCGAGAGCTGCGGCGCGCACTTTTACCGTGGTGTCGATGTCAAACCGTTTTACATCAAGAAACCTGTTGACAATCTCTTCGCCCTGATGCTGATATTAAATCGGCTACGGGGTTGGGGAGTTGTCGGAGGTATGTCAGATCCACGCCTCTACAAGGTGTGGGTACGGCTCTCCTCCCAGGTGCCTTCGATGTTCTTCGGTGGGACGGACCTCGCTGCCGACTACTACGTAGTCAGCCCGCCTACGGCAGTCTCGGTATACACCAAGACTCCGTACGGGCGGCTGCTCGCGGATACCCGTACCTCGGGTTTCCGTCTTGCTCGTATCGCTCGAGAACGCAAGTTCTTCAGCGAAAAGCACGACAGTGGTCGCTACATAGCGTGGTTCCATACTGGAGGTGAAATCACCGACAGCATGAAGTCCGCCGGCGTGCGCGTTATACGCACTTCGGAGTGGCTAACGCCGGTTCCCACATTCCCTCAGGAGTGTGGGCCAGCGAGCTCTCCTCGGTAA |
| **Qβrep-His6 insert** |
| Start Stop  ATGTCTAAGACAGCATCTTCGCGTAACTCTCTCAGCGCACAATTGCGCCGAGCCGCGAACACAAGAATTGAGGTTGAAGGTAACCTCGCACTTTCCATTGCCAACGATTTACTGTTGGCCTATGGTCAGTCGCCATTTAACTCTGAGGCTGAGTGTATTTCATTCAGCCCGAGATTCGACGGGACCCCGGATGACTTTAGGATAAATTATCTTAAAGCCGAGATCATGTCGAAGTATGACGACTTCAGCCTAGGTATTGATACCGAAGCTGTTGCCTGGGAGAAGTTCCTGGCAGCAGAGGCTGAATGTGCTTTAACGAACGCTCGTCTCTATAGGCCTGACTACAGTGAGGATTTCAATTTCTCACTGGGCGAGTCATGTATACACATGGCTCGTAGAAAAATAGCCAAGCTAATAGGAGATGTTCCGTCCGTTGAGGGTATGTTGCGTCACTGCCGATTTTCTGGCGGTGCTACAACAACGAATAACCGTTCGTACGGTCATCCGTCCTTCAAGTTTGCGCTTCCGCAAGCGTGTACGCCTCGGGCTTTGAAGTATGTTTTAGCTCTCAGAGCTTCTACACATTTCGATATCAGAATTTCTGATATTAGCCCTTTTAATAAAGCAGTTACTGTACCTAAGAACAGTAAGACAGATCGTTGTATTGCTATCGAACCTGGTTGGAATATGTTTTTCCAACTGGGTATCGGTGGCATTCTACGCGATCGGTTGCGTTGCTGGGGTATCGATCTGAATGATCAGACGATAAATCAGCGCCGCGCTCACGAAGGCTCCGTTACTAATAACTTAGCAACGGTTGATCTCTCAGCGGCAAGCGATTCTATATCTCTTGCCCTCTGTGAGCTCTTATTGCCCCCAGGCTGGTTTGAGGTTCTTATGGACCTCAGATCACCTAAGGGGCGATTGCCTGACGGTAGTGTTGTTACCTACGAGAAGATTTCTTCTATGGGTAACGGTTACACATTCGAGCTCGAGTCGCTTATTTTTGCTTCTCTCGCTCGTTCCGTTTGTGAGATACTGGACTTAGACTCGTCTGAGGTCACTGTTTACGGAGACGATATTATTTTACCGTCCTGTGCAGTCCCTGCCCTCCGGGAAGTTTTTAAGTATGTTGGTTTTACGACCAATACTAAAAAGACTTTTTCCGAGGGGCCGTTCAGAGAGTCGTGCGGCAAGCACTACTATTCTGGCGTAGATGTTACTCCCTTTTACATACGTCACCGTATAGTGAGTCCTGCCGATTTAATACTGGTTTTGAATAACCTATATCGGTGGGCCACAATTGACGGCGTATGGGATCCTAGGGCCCATTCTGTGTACCTCAAGTATCGTAAGTTGCTGCCTAAACAGCTGCAACGTAATACTATACCTGATGGTTACGGTGATGGTGCCCTCGTCGGATCGGTCCTAATCAATCCTTTCGCGAAAAACCGCGGGTGGATCCGGTACGTACCGGTGATTACGGACCATACAAGGGACCGAGAGCGCGCTGAGTTGGGGTCGTATCTCTACGACCTCTTCTCGCGTTGTCTCTCGGAAAGTAACGATGGGTTGCCTCTTAGGGGTCCATCGGGTTGCGATTCTGCGGATCTATTTGCCATCGATCAGCTTATCTGTAGGAGTAATCCTACGAAGATAAGCAGGTCTACCGGCAAATTCGATATACAGTATATCGCGTGCAGTAGCCGTGTTCTGGCACCCTACGGGGTCTTCCAGGGCACGAAGGTTGCGTCTCTACACGAGGCGCACCACCACCACCACCACTAA |
| **EF-Tu-His6 insert** |
| Start Stop  ATGTCTAAAGAAAAGTTTGAACGTACAAAACCGCACGTTAACGTCGGTACTATCGGCCACGTTGACCATGGTAAAACAACGCTGACCGCTGCAATCACTACCGTACTGGCTAAAACCTACGGCGGTGCTGCTCGCGCATTCGACCAGATCGATAACGCGCCGGAAGAAAAAGCTCGTGGTATCACCATCAACACTTCTCACGTTGAATACGACACCCCGACCCGTCACTACGCACACGTAGACTGCCCGGGGCACGCCGACTATGTTAAAAACATGATCACCGGTGCTGCGCAGATGGACGGCGCGATCCTGGTAGTTGCTGCGACTGACGGCCCGATGCCGCAGACTCGTGAGCACATCCTGCTGGGTCGTCAGGTAGGCGTTCCGTACATCATCGTGTTCCTGAACAAATGCGACATGGTTGATGACGAAGAGCTGCTGGAACTGGTTGAAATGGAAGTTCGTGAACTTCTGTCTCAGTACGACTTCCCGGGCGACGACACTCCGATCGTTCGTGGTTCTGCTCTGAAAGCGCTGGAAGGCGACGCAGAGTGGGAAGCGAAAATCCTGGAACTGGCTGGCTTCCTGGATTCTTACATTCCGGAACCAGAGCGTGCGATTGACAAGCCGTTCCTGCTGCCGATCGAAGACGTATTCTCCATCTCCGGTCGTGGTACCGTTGTTACCGGTCGTGTAGAACGCGGTATCATCAAAGTTGGTGAAGAAGTTGAAATCGTTGGTATCAAAGAGACTCAGAAGTCTACCTGTACTGGCGTTGAAATGTTCCGCAAACTGCTGGACGAAGGCCGTGCTGGTGAGAACGTAGGTGTTCTGCTGCGTGGTATCAAACGTGAAGAAATCGAACGTGGTCAGGTACTGGCTAAGCCGGGCACCATCAAGCCGCACACCAAGTTCGAATCTGAAGTGTACATTCTGTCCAAAGATGAAGGCGGCCGTCATACTCCGTTCTTCAAAGGCTACCGTCCGCAGTTCTACTTCCGTACTACTGACGTGACTGGTACCATCGAACTGCCGGAAGGCGTAGAGATGGTAATGCCGGGCGACAACATCAAAATGGTTGTTACCCTGATCCACCCGATCGCGATGGACGACGGTCTGCGTTTCGCAATCCGTGAAGGCGGCCGTACCGTTGGCGCGGGCGTTGTAGCAAAAGTTCTGCACCACCACCACCACCACTAA |
| **EF-Ts-His6 insert** |
| Start Stop  ATGGCTGAAATTACCGCATCCCTGGTAAAAGAGCTGCGTGAGCGTACTGGCGCAGGCATGATGGATTGCAAAAAAGCACTGACTGAAGCTAACGGCGACATCGAGCTGGCAATCGAAAACATGCGTAAGTCCGGTGCTATTAAAGCAGCGAAAAAAGCAGGCAACGTTGCTGCTGACGGCGTGATCAAAACCAAAATCGACGGCAACTACGGCATCATTCTGGAAGTTAACTGCCAGACTGACTTCGTTGCAAAAGACGCTGGTTTCCAGGCGTTCGCAGACAAAGTTCTGGACGCAGCTGTTGCTGGCAAAATCACTGACGTTGAAGTTCTGAAAGCACAGTTCGAAGAAGAACGTGTTGCGCTGGTAGCGAAAATTGGTGAAAACATCAACATTCGCCGCGTTGCTGCGCTGGAAGGCGACGTTCTGGGTTCTTATCAGCACGGTGCGCGTATCGGCGTTCTGGTTGCTGCTAAAGGCGCTGACGAAGAGCTGGTTAAACACATCGCTATGCACGTTGCTGCAAGCAAGCCAGAATTCATCAAACCGGAAGACGTATCCGCTGAAGTGGTAGAAAAAGAATACCAGGTACAGCTGGATATCGCGATGCAGTCTGGTAAGCCGAAAGAAATCGCGGAGAAAATGGTTGAAGGCCGCATGAAGAAATTCACCGGCGAAGTTTCTCTGACCGGTCAGCCGTTCGTTATGGAACCAAGCAAAACTGTTGGTCAGCTGCTGAAAGAGCATAACGCTGAAGTGACTGGCTTCATCCGCTTCGAAGTGGGTGAAGGCATCGAGAAAGTTGAGACTGACTTTGCAGCAGAAGTTGCTGCGATGTCCAAGCAGTCTCACCACCACCACCACCACTAA |
| **S1-His6 insert** |
| Start Stop  ATGACTGAATCTTTTGCTCAACTCTTTGAAGAGTCCTTAAAAGAAATCGAAACCCGCCCGGGTTCTATCGTTCGTGGCGTTGTTGTTGCTATCGACAAAGACGTAGTACTGGTTGACGCTGGTCTGAAATCTGAGTCCGCCATCCCGGCTGAGCAGTTCAAAAACGCCCAGGGCGAGCTGGAAATCCAGGTAGGTGACGAAGTTGACGTTGCTCTGGACGCAGTAGAAGACGGCTTCGGTGAAACTCTGCTGTCCCGTGAGAAAGCTAAACGTCACGAAGCCTGGATCACGCTGGAAAAAGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATCAACGGCAAAGTTAAGGGCGGCTTCACTGTTGAGCTGAACGGTATTCGTGCGTTCCTGCCAGGTTCTCTGGTAGACGTTCGTCCGGTGCGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAATTTAAAGTAATCAAGCTGGATCAGAAGCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCGAATCCGAAAACAGCGCAGAGCGCGATCAGCTGCTGGAAAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGTTAAGAACCTCACTGACTACGGTGCATTCGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACATCACTGACATGGCCTGGAAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGAAATCACTGTTAAAGTGCTGAAGTTCGACCGCGAACGTACCCGTGTATCCCTGGGCCTGAAACAGCTGGGCGAAGATCCGTGGGTAGCTATCGCTAAACGTTATCCGGAAGGTACCAAACTGACTGGTCGCGTGACCAACCTGACCGACTACGGCTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACACGTTTCCGAAATGGACTGGACCAACAAAAACATCCACCCGTCCAAAGTTGTTAACGTTGGCGATGTAGTGGAAGTTATGGTTCTGGATATCGACGAAGAACGTCGTCGTATCTCCCTGGGTCTGAAACAGTGCAAAGCTAACCCGTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCGTGTTGAAGGTAAAATCAAGTCTATCACTGACTTCGGTATCTTCATCGGCTTGGACGGCGGCATCGACGGCCTGGTTCACCTGTCTGACATCTCCTGGAACGTTGCAGGCGAAGAAGCAGTTCGTGAATACAAAAAAGGCGACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAACGTATCTCCCTGGGCGTTAAACAGCTCGCAGAAGATCCGTTCAACAACTGGGTTGCTCTGAACAAGAAAGGCGCTATCGTAACCGGTAAAGTAACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGGCTGACGGCGTTGAAGGTTACCTGCGTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCTACCCTGGTTCTGAGCGTTGGCGACGAAGTTGAAGCTAAATTCACCGGCGTTGATCGTAAAAACCGCGCAATCAGCCTGTCTGTTCGTGCGAAAGACGAAGCTGACGAGAAAGATGCAATCGCAACTGTTAACAAACAGGAAGATGCAAACTTCTCCAACAACGCAATGGCTGAAGCTTTCAAAGCAGCTAAAGGCGAGCACCACCACCACCACCACCACTAA |
| **MTF-His6 insert** |
| Start Stop  ATGTCAGAATCACTACGTATTATTTTTGCGGGTACACCTGACTTTGCAGCGCGTCATCTCGACGCGCTGTTGTCTTCTGGTCATAACGTCGTTGGCGTGTTCACCCAGCCAGACCGACCGGCAGGACGCGGTAAAAAACTGATGCCCAGCCCGGTTAAAGTTCTGGCTGAGGAAAAAGGTCTGCCCGTTTTTCAACCTGTTTCCCTGCGTCCACAAGAAAACCAGCAACTGGTCGCCGAACTGCAAGCTGATGTTATGGTCGTCGTCGCCTATGGTTTAATTCTGCCGAAAGCAGTGCTGGAGATGCCGCGTCTTGGCTGTATCAACGTTCATGGTTCACTGCTGCCACGCTGGCGCGGTGCTGCACCAATCCAACGCTCACTATGGGCGGGTGATGCAGAAACTGGTGTGACCATTATGCAAATGGATGTCGGTTTAGACACCGGTGATATGCTCTATAAGCTCTCCTGCCCGATTACCGCAGAAGATACCAGTGGTACGCTGTACGACAAGCTGGCAGAGCTTGGCCCACAAGGGCTTATCACCACGTTGAAACAACTGGCAGACGGCACGGCGAAACCAGAAGTTCAGGACGAAACTCTTGTCACTTACGCCGAGAAGTTGAGTAAAGAAGAAGCGCGTATTGACTGGTCACTTTCGGCAGCACAGCTTGAACGCTGCATTCGCGCTTTCAATCCATGGCCAATGAGCTGGCTGGAAATTGAAGGACAGCCGGTTAAAGTCTGGAAAGCATCGGTCATTGATACGGCAACCAACGCTGCACCAGGAACGATCCTTGAAGCCAACAAACAAGGCATTCAGGTTGCGACTGGTGATGGCATCCTGAACCTGCTCTCGTTACAACCTGCGGGTAAGAAAGCGATGAGCGCGCAAGACCTCCTGAACTCTCGTCGGGAATGGTTTGTTCCGGGCAACCGTCTGGTCCACCACCACCACCACCACTAA |
| **Ile-His6 insert** |
| Start Stop  ATGAGTGACTATAAATCAACCCTGAATCTGCCGGAAACAGGGTTCCCGATGCGTGGCGATCTCGCCAAGCGCGAACCCGGAATGCTGGCGCGTTGGACTGATGATGATCTGTACGGCATCATCCGTGCGGCTAAAAAAGGCAAAAAAACCTTCATTCTGCATGATGGCCCTCCTTATGCGAATGGCAGCATTCATATTGGTCACTCGGTTAACAAGATTCTGAAAGACATTATCGTGAAGTCCAAAGGGCTTTCCGGTTATGACTCGCCGTATGTGCCTGGCTGGGACTGCCACGGTCTGCCGATCGAGCTGAAAGTCGAGCAAGAATACGGTAAGCCGGGTGAGAAGTTCACCGCCGCCGAGTTCCGCGCCAAGTGCCGCGAATACGCGGCGACCCAGGTTGACGGTCAACGCAAAGACTTTATCCGTCTGGGCGTGCTGGGCGACTGGTCGCACCCGTACCTGACCATGGACTTCAAAACTGAAGCCAACATCATCCGCGCGCTGGGCAAAATCATCGGCAACGGTCACCTGCACAAAGGCGCGAAGCCAGTTCACTGGTGCGTTGACTGCCGTTCTGCGCTGGCGGAAGCGGAAGTTGAGTATTACGACAAAACTTCTCCGTCCATCGACGTTGCTTTCCAGGCAGTCGATCAGGATGCACTGAAAGCAAAGTTTGCCGTAAGCAACGTTAACGGCCCAATCTCGCTGGTAATCTGGACCACCACGCCGTGGACTCTGCCTGCCAACCGCGCAATCTCTATTGCACCAGATTTCGACTATGCGCTGGTGCAGATCGACGGTCAGGCCGTGATTCTGGCGAAAGATCTGGTTGAAAGCGTAATGCAGCGTATCGGCGTGACCGATTACACCATTCTCGGCACGGTAAAAGGTGCGGAGCTTGAGCTGCTGCGCTTTACCCATCCGTTTATGGGCTTCGACGTTCCGGCAATCCTCGGCGATCACGTTACCCTGGATGCCGGTACCGGTGCCGTTCACACCGCGCCTGGCCACGGCCCGGACGACTATGTGATCGGTCAGAAATACGGCCTGGAAACCGCTAACCCGGTTGGCCCGGACGGCACTTATCTGCCGGGCACTTATCCGACGCTGGATGGCGTGAACGTCTTCAAAGCGAACGACATCGTCGTTGCGCTGCTGCAAGAAAAAGGCGCGCTGCTGCACGTTGAGAAAATGCAGCACAGCTATCCGTGCTGCTGGCGTCACAAAACGCCGATCATCTTCCGCGCGACGCCGCAGTGGTTCGTCAGCATGGATCAGAAAGGTCTGCGTGCGCAGTCACTGAAAGAGATCAAAGGCGTGCAGTGGATCCCGGACTGGGGCCAGGCGCGTATCGAGTCGATGGTTGCTAACCGTCCTGACTGGTGTATCTCCCGTCAGCGCACCTGGGGTGTACCGATGTCACTGTTCGTGCACAAAGACACGGAAGAGCTGCATCCGCGTACCCTTGAACTGATGGAAGAAGTGGCAAAACGCGTTGAAGTCGATGGCATCCAGGCGTGGTGGGATCTCGATGCGAAAGAGATCCTCGGCGACGAAGCTGATCAGTACGTGAAAGTGCCGGACACATTGGATGTATGGTTTGACTCCGGATCTACCCACTCTTCTGTTGTTGACGTGCGTCCGGAGTTTGCCGGTCACGCAGCGGACATGTATCTGGAAGGTTCTGACCAACACCGCGGCTGGTTCATGTCTTCCCTAATGATCTCCACCGCGATGAAGGGTAAAGCGCCGTATCGTCAGGTACTGACCCACGGCTTTACCGTGGATGGTCAGGGCCGCAAGATGTCTAAATCCATCGGCAATACCGTTTCGCCGCAGGATGTGATGAACAAACTGGGCGCGGATATTCTGCGTCTGTGGGTGGCATCAACCGACTACACCGGTGAAATGGCCGTTTCTGACGAGATCCTGAAACGTGCTGCCGATAGCTATCGTCGTATCCGTAACACCGCGCGCTTCCTGCTGGCAAACCTGAACGGTTTTGATCCAGCAAAAGATATGGTGAAACCGGAAGAGATGGTGGTACTGGATCGCTGGGCCGTAGGTTGTGCGAAAGCGGCACAGGAAGACATCCTCAAGGCGTACGAAGCATACGATTTCCACGAAGTGGTACAGCGTCTGATGCGCTTCTGCTCCGTTGAGATGGGTTCCTTCTACCTCGACATCATCAAAGACCGTCAGTACACCGCCAAAGCGGACAGTGTGGCGCGTCGTAGCTGCCAGACTGCGCTATATCACATCGCAGAAGCGCTGGTGCGCTGGATGGCACCAATCCTCTCCTTCACCGCTGATGAAGTGTGGGGCTACCTGCCGGGCGAACGTGAAAAATACGTCTTCACCGGTGAGTGGTACGAAGGCCTGTTTGGCCTGGCAGACAGTGAAGCGATGAACGATGCGTTCTGGGACGAGCTGTTGAAAGTGCGTGGCGAAGTGAACAAAGTCATTGAGCAAGCGCGTGCCGACAAGAAAGTGGGTGGCTCGCTGGAAGCGGCAGTAACCTTGTATGCAGAACCGGAACTGTCGGCGAAACTGACCGCGCTGGGCGATGAATTACGATTTGTCCTGTTGACCTCCGGCGCTACCGTTGCAGACTATAACGACGCACCTGCTGATGCTCAGCAGAGCGAAGTACTCAAAGGGCTGAAAGTCGCGTTGAGTAAAGCCGAAGGTGAGAAGTGCCCACGCTGCTGGCACTACACCCAGGATGTCGGCAAGGTGGCGGAACACGCAGAAATCTGTGGCCGCTGTGTCAGCAACGTCGCCGGTGACGGTGAAAAACGTAAGTTTGCCCACCACCACCACCACCACTAA |
| **His6-IF1 insert** |
| Start Stop  ATGCATCACCATCACCATCACGCGAAAGAAGATAATATTGAAATGCAAGGCACGGTCCTCGAAACGCTTCCGAACACGATGTTCCGGGTCGAGCTCGAAAACGGCCACGTCGTGACGGCGCATATCTCGGGCAAAATGCGCAAAAACTACATCCGGATCCTGACCGGCGACAAGGTCACCGTCGAACTCACGCCGTACGACCTCTCGAAAGGCCGCATCGTCTTCCGCTCGCGCTAA |
| **His6-IF3 insert** |
| Start Stop  ATGCATCACCATCACCATCACAAAGGCGGAAAACGAGTTCAAACGGCGCGCCCTAACCGTATCAATGGCGAAATTCGCGCCCAGGAAGTTCGCTTAACAGGTCTGGAAGGCGAGCAGCTTGGTATTGTGAGTCTGAGAGAAGCTCTGGAGAAAGCAGAAGAAGCCGGAGTAGACTTAGTCGAGATCAGCCCTAACGCCGAGCCGCCGGTTTGTCGTATAATGGATTACGGCAAATTCCTCTATGAAAAGAGCAAGTCTTCTAAGGAACAGAAGAAAAAGCAAAAAGTTATCCAGGTTAAGGAAATTAAATTCCGTCCTGGTACAGATGAAGGCGACTATCAGGTAAAACTCCGCAGCCTGATTCGCTTTCTCGAAGAGGGTGATAAAGCCAAAATCACGCTGCGTTTCCGCGGTCGTGAGATGGCGCACCAGCAAATCGGTATGGAAGTGCTTAATCGCGTGAAAGACGATTTGCAAGAACTGGCAGTGGTCGAATCCTTCCCAACGAAGATCGAAGGCCGCCAGATGATCATGGTGCTCGCTCCTAAGAAGAAACAGTAA |
| **His6-AlaRS insert** |
| Start Stop  ATGCACCACCACCACCACCACAGCAAGAGCACCGCTGAGATCCGTCAGGCGTTTCTCGACTTTTTCCATAGTAAGGGACATCAGGTAGTTGCCAGCAGCTCCCTGGTACCCCATAACGACCCAACTTTGTTGTTTACCAACGCCGGGATGAACCAGTTCAAGGATGTGTTCCTTGGGCTCGACAAGCGTAATTATTCCCGCGCTACCACTTCCCAACGCTGCGTGCGTGCGGGTGGTAAACACAACGACCTGGAAAACGTCGGTTACACCGCGCGTCACCATACCTTCTTCGAAATGCTGGGCAACTTCAGCTTCGGCGACTATTTCAAACACGATGCCATTCAGTTTGCATGGGAACTGCTGACCAGCGAAAAATGGTTTGCCCTGCCGAAAGAGCGTCTGTGGGTTACCGTCTATGAAAGCGACGACGAAGCCTACGAAATCTGGGAAAAAGAAGTAGGGATCCCGCGCGAACGTATTATTCGCATCGGCGATAACAAAGGTGCGCCATACGCATCTGACAACTTCTGGCAGATGGGTGACACTGGTCCGTGCGGCCCGTGCACCGAAATCTTCTACGATCACGGCGACCACATTTGGGGGGGCCCTCCGGGAAGCCCGGAAGAAGACGGCGACCGCTACATTGAGATCTGGAACATCGTCTTCATGCAGTTCAACCGCCAGGCCGATGGCACGATGGAACCGCTGCCGAAGCCGTCTGTAGATACCGGTATGGGTCTGGAGCGTATTGCTGCGGTGCTGCAACACGTTAACTCTAACTATGACATCGACCTGTTCCGCACGCTGATCCAGGCGGTAGCGAAAGTCACTGGCGCAACCGATCTGAGCAATAAATCGCTGCGCGTAATCGCTGACCACATTCGTTCTTGTGCGTTCCTGATCGCGGATGGCGTAATGCCGTCCAATGAAAACCGTGGTTATGTACTGCGTCGTATCATTCGTCGCGCAGTGCGTCACGGTAATATGCTCGGCGCGAAAGAAACCTTCTTCTACAAACTGGTTGGTCCGCTGATCGACGTTATGGGCTCTGCGGGTGAAGACCTGAAACGCCAGCAGGCGCAGGTTGAGCAGGTGCTGAAGACTGAAGAAGAGCAGTTTGCTCGTACTCTGGAGCGCGGTCTGGCGTTGCTGGATGAAGAGCTGGCAAAACTTTCTGGTGATACGCTGGATGGTGAAACTGCTTTCCGTCTGTACGACACCTATGGCTTCCCGGTTGACCTGACGGCTGATGTTTGTCGTGAGCGCAACATCAAAGTTGACGAAGCTGGTTTTGAAGCTGCAATGGAAGAGCAGCGTCGTCGCGCGCGCGAAGCCAGCGGCTTTGGTGCCGATTACAACGCAATGATCCGTGTTGACAGTGCATCTGAGTTTAAAGGCTATGACCATCTGGAACTGAACGGCAAAGTGACTGCGCTGTTTGTTGATGGTAAAGCGGTTGATGCCATCAATGCAGGCCAGGAAGCTGTGGTCGTGCTGGATCAAACGCCATTCTATGCGGAATCCGGCGGTCAGGTTGGCGATAAAGGCGAACTGAAAGGCGCTAACTTCTCCTTTGCGGTGGAAGATACGCAGAAATACGGCCAGGCGATTGGTCACATCGGTAAACTTGCTGCGGGTTCTCTGAAAGTGGGCGACGCGGTGCAGGCTGATGTTGATGAGGCTCGTCGCGCCCGTATTCGTCTGAATCACTCCGCAACGCACCTGATGCACGCTGCGCTGCGCCAGGTTCTGGGTACTCATGTATCGCAGAAAGGTTCACTGGTTAACGACAAGGTGCTGCGCTTCGACTTCTCACACAACGAAGCGATGAAACCAGAAGAGATTCGTGCGGTCGAAGACCTGGTGAACACACAGATTCGTCGCAATTTGCCGATCGAAACCAACATCATGGATCTCGAAGCGGCGAAAGCGAAAGGTGCGATGGCGCTGTTCGGCGAGAAGTATGATGAGCGCGTACGCGTGCTGAGCATGGGCGATTTCTCTACCGAGTTGTGTGGCGGTACTCACGCCAGCCGCACTGGTGATATTGGTCTGTTCCGCATCATCTCTGAATCGGGTACTGCTGCCGGCGTTCGTCGTATCGAAGCGGTAACCGGAGAAGGTGCTATCGCCACCGTTCATGCAGACAGCGATCGCTTAAGCGAAGTCGCGCATCTGCTGAAAGGCGATAGCAATAATCTGGCTGATAAAGTGCGCTCAGTACTGGAACGTACGCGTCAGCTGGAAAAAGAGTTACAACAGCTTAAAGAACAAGCTGCCGCACAGGAGAGCGCAAATCTTTCCAGTAAGGCAATCGATGTTAATGGTGTTAAGCTGTTGGTTAGCGAGCTTAGCGGTGTTGAGCCGAAAATGTTGCGTACCATGGTTGACGATTTAAAAAATCAGCTGGGGTCGACAATTATCGTGCTGGCAACGGTAGTCGAAGGTAAGGTTTCTCTGATTGCAGGCGTATCTAAGGACGTCACAGATCGTGTGAAAGCAGGGGAACTGATTGGTATGGTCGCTCAGCAGGTGGGCGGCAAGGGTGGTGGACGTCCTGACATGGCGCAAGCCGGTGGTACGGATGCTGCGGCCTTACCGGCAGCGTTAGCCAGTGTGAAAGGCTGGGTCAGCGCGAAATTGCAATAA |
| **His6-AsnRS insert** |
| Start Stop  ATGCACCACCACCACCACCACAGCGTTGTGCCTGTAGCCGACGTACTCCAGGGCCGTGTAGCCGTTGACAGCGAAGTCACCGTGCGCGGATGGGTACGTACCCGCCGAGATTCAAAAGCTGGCATCTCCTTCCTCGCCGTTTATGACGGTTCCTGCTTTGATCCTGTACAGGCTGTCATCAATAATTCTCTGCCCAATTACAATGAAGACGTCCTGCGTCTGACCACCGGCTGCTCGGTCATTGTGACGGGTAAAGTCGTGGCGTCGCCGGGCCAGGGGCAACAATTTGAAATCCAGGCCAGCAAGGTTGAAGTTGCTGGTTGGGTTGAAGATCCAGACACTTACCCGATGGCGGCAAAACGCCACAGCATTGAGTATCTGCGTGAAGTCGCTCACCTGCGTCCGCGCACAAACCTGATTGGTGCCGTCGCGCGCGTTCGCCATACGCTGGCGCAGGCGCTGCATCGCTTCTTTAACGAGCAGGGATTCTTCTGGGTTTCAACGCCACTGATTACCGCATCTGATACCGAAGGTGCAGGCGAAATGTTCCGCGTTTCTACGCTGGATCTGGAAAACCTGCCGCGTAACGATCAGGGCAAAGTGGATTTCGACAAAGACTTCTTTGGTAAAGAGTCTTTCCTGACCGTATCTGGCCAGTTGAACGGCGAAACCTACGCTTGCGCATTGTCCAAAATCTATACCTTCGGCCCGACTTTCCGTGCTGAAAACTCCAACACCAGCCGTCACCTGGCGGAGTTCTGGATGCTGGAGCCGGAAGTGGCGTTTGCTAACCTGAACGATATTGCGGGTCTGGCTGAAGCCATGCTGAAATATGTCTTCAAAGCGGTTCTCGAAGAACGCGCTGACGACATGAAGTTCTTCGCTGAACGCGTAGATAAAGATGCCGTTTCACGTCTGGAACGCTTCATTGAAGCCGATTTTGCGCAGGTGGATTATACCGACGCAGTGACCATTCTCGAAAACTGCGGCAGGAAGTTTGAAAACCCGGTTTACTGGGGAGTCGATCTCTCTTCTGAGCATGAGCGTTATCTGGCGGAAGAACACTTTAAAGCACCGGTAGTGGTTAAAAACTATCCGAAAGATATTAAAGCGTTCTATATGCGCCTTAACGAAGACGGTAAAACCGTTGCGGCTATGGACGTTCTGGCTCCGGGCATCGGTGAGATCATTGGTGGCTCCCAGCGTGAAGAACGTCTGGACGTGCTGGACGAGCGTATGCTGGAAATGGGCCTGAATAAAGAAGATTACTGGTGGTATCGCGATCTGCGTCGCTACGGTACTGTTCCGCATTCAGGTTTCGGTCTTGGTTTTGAACGTCTGATTGCTTACGTAACTGGCGTGCAAAACGTACGTGATGTGATTCCGTTCCCACGTACTCCGCGTAACGCCAGCTTCTAA |
| **His6-PheRSα + PheRSβ insert** |
| Start Stop PheRSα PheRSβ  ATGCACCACCACCACCACTCACATCTCGCAGAACTGGTTGCCAGTGCGAAGGCGGCCATTAGCCAGGCGTCAGATGTTGCCGCGTTAGATAATGTGCGCGTCGAATATTTGGGTAAAAAAGGGCACTTAACCCTTCAGATGACGACCCTGCGTGAGCTGCCGCCAGAAGAGCGTCCGGCAGCTGGTGCGGTTATCAACGAAGCGAAAGAGCAGGTTCAGCAGGCGCTGAATGCGCGTAAAGCGGAACTGGAAAGCGCTGCACTGAATGCGCGTCTGGCGGCGGAAACGATTGATGTCTCTCTGCCAGGTCGTCGCATTGAAAACGGCGGTCTGCATCCGGTTACCCGTACCATCGACCGTATCGAAAGTTTCTTCGGTGAGCTTGGCTTTACCGTGGCAACCGGGCCGGAAATCGAAGACGATTATCATAACTTCGATGCTCTGAACATTCCTGGTCACCACCCGGCGCGCGCTGACCACGACACTTTCTGGTTTGACACTACCCGCCTGCTGCGTACCCAGACCTCTGGCGTACAGATCCGCACCATGAAAGCCCAGCAGCCACCGATTCGTATCATCGCGCCTGGCCGTGTTTATCGTAACGACTACGACCAGACTCACACGCCGATGTTCCATCAGATGGAAGGTCTGATTGTTGATACCAACATCAGCTTTACCAACCTGAAAGGCACGCTGCACGACTTCCTGCGTAACTTCTTTGAGGAAGATTTGCAGATTCGCTTCCGTCCTTCCTACTTCCCGTTTACCGAACCTTCTGCGGAAGTGGACGTCATGGGTAAAAACGGTAAATGGCTGGAAGTGCTGGGCTGCGGGATGGTGCATCCGAACGTGTTGCGTAACGTTGGCATCGACCCGGAAGTTTACTCTGGTTTCGCCTTCGGGATGGGGATGGAGCGTCTGACTATGTTGCGTTACGGCGTCACCGACCTGCGTTCATTCTTCGAAAACGATCTGCGTTTCCTCAAACAGTTTAAATAAGGCAGGAATAGATTATGAAGTTCAGTGAACTGTGGTTACGCGAATGGGTGAACCCGGCGATTGATAGCGATGCGCTGGCAAATCAAATCACTATGGCGGGCCTGGAAGTTGACGGTGTAGAACCGGTTGCCGGCAGCTTCCACGGCGTGGTCGTTGGTGAAGTGGTTGAGTGTGCGCAGCATCCGAACGCTGACAAACTGCGTGTGACAAAAGTGAATGTCGGCGGCGATCGCCTGCTGGACATCGTCTGCGGTGCGCCAAACTGCCGTCAGGGCCTGCGTGTAGCGGTAGCGACCATTGGTGCTGTTCTGCCGGGTGATTTCAAAATTAAAGCGGCGAAACTGCGTGGCGAACCGTCTGAAGGGATGCTGTGCTCCTTCTCTGAACTGGGCATTTCTGACGATCACAGCGGCATTATCGAACTGCCTGCGGATGCGCCGATTGGCACCGATATCCGTGAATACCTGAAACTTGATGACAACACCATCGAAATCAGCGTGACGCCAAACCGTGCCGACTGCTTAGGCATCATTGGTGTTGCGCGTGACGTTGCCGTGCTGAACCAGCTGCCGCTGGTTCAACCGGAAATCGTTCCGGTTGGTGCGACCATCGACGACACGCTGCCGATTACAGTCGAAGCGCCGGAAGCCTGCCCGCGTTATCTTGGCCGTGTGGTAAAAGGCATTAACGTTAAAGCGCCAACTCCGCTGTGGATGAAAGAAAAACTGCGTCGTTGCGGGATCCGTTCTATCGATGCAGTTGTTGACGTCACCAACTATGTGCTGCTCGAACTGGGCCAGCCGATGCACGCTTTCGATAAAGATCGCATTGAAGGCGGCATTGTGGTGCGGATGGCGAAAGAGGGCGAAACGCTGGTGCTGCTCGACGGTACTGAAGCGAAGCTGAATGCTGACACTCTGGTCATCGCCGACCACAACAAGGCGCTGGCGATGGGCGGCATCTTCGGTGGCGAACACTCTGGCGTGAATGACGAAACACAAAACGTGCTGCTGGAATGCGCGTTCTTTAGCCCGCTGTCTATCACCGGTCGTGCTCGTCGTCATGGCCTGCATACCGATGCGTCTCACCGTTATGAGCGTGGCGTTGATCCGGCACTGCAACACAAAGCGATGGAACGTGCGACCCGTCTGCTGATCGACATCTGCGGTGGTGAGGCTGGCCCGGTAATTGATATCACCAACGAAGCAACGCTGCCGAAGCGTGCAACCATCACTCTACGTCGTAGCAAACTGGATCGCCTGATCGGCCATCATATTGCGGATGAGCAGGTAACTGACATTCTGCGTCGTCTCGGCTGCGAAGTGACCGAAGGCAAAGACGAATGGCAGGCAGTTGCGCCGAGCTGGCGTTTCGATATGGAGATTGAAGAAGATCTGGTTGAAGAAGTCGCGCGTGTTTACGGCTACAACAACATCCCGGATGAGCCGGTACAGGCAAGCCTGATTATGGGTACTCACCGTGAAGCTGACCTGTCGCTCAAGCGCGTGAAAACGCTGCTCAACGACAAAGGCTATCAGGAAGTGATCACCTACAGCTTCGTTGATCCGAAAGTGCAGCAGATGATCCATCCAGGCGTTGAAGCCTTACTGCTGCCAAGCCCGATCTCTGTTGAAATGTCAGCAATGCGTCTTTCTCTGTGGACTGGCCTGCTGGCAACCGTGGTGTACAACCAGAACCGTCAGCAGAACCGTGTGCGCATTTTCGAAAGCGGTCTGCGTTTCGTACCAGATACTCAGGCACCGTTGGGCATTCGTCAGGATCTGATGTTAGCCGGTGTGATTTGCGGTAACCGTTACGAAGAGCACTGGAACCTGGCAAAAGAGACCGTTGATTTCTATGATTTGAAAGGCGATCTTGAATCCGTTCTCGACCTGACCGGTAAACTGAATGAGGTTGAGTTCCGTGCAGAAGCGAATCCGGCACTGCATCCGGGGCAATCCGCAGCGATTTATCTGAAAGGTGAACGTATTGGTTTTGTTGGGGTTGTTCATCCTGAACTGGAACGTAAACTGGATCTTAACGGTCGCACTCTGGTGTTCGAACTGGAGTGGAACAAGCTCGCAGACCGCGTGGTGCCTCAGGCGCGCGAGATTTCTCGCTTCCCGGCGAACCGTCGTGACATCGCGGTGGTGGTCGCAGAAAACGTTCCCGCAGCGGATATTTTATCCGAATGTAAGAAAGTTGGCGTAAATCAGGTAGTTGGCGTAAACTTATTTGACGTGTACCGCGGTAAGGGTGTTGCGGAGGGGTATAAGAGCCTCGCCATAAGCCTGATCCTGCAAGATACCAGCCGTACACTCGAAGAAGAGGAGATTGCCGCTACCGTCGCCAAATGTGTAGAGGCATTAAAAGAGCGATTCCAGGCATCATTGAGGGATTAA |

Supplementary Table 4: Composition of in-house PURE

|  |  |  |
| --- | --- | --- |
| **Component** | **Stock** | **Final** |
| **LD1 (10 x)** | | |
| RF1 | 10 x | 1 x |
| HisRS | 10 x | 1 x |
| TyrRS | 10 x | 1 x |
| CysRS | 10 x | 1 x |
| TrpRS | 10 x | 1 x |
| SerRS | 10 x | 1 x |
| ValRS | 10 x | 1 x |
| MetRS | 10 x | 1 x |
| ArgRS | 10 x | 1 x |
| GlnRS | 10 x | 1 x |
| LeuRS | 10 x | 1 x |
| ThrRS | 10 x | 1 x |
| LysRS | 10 x | 1 x |
| **LD2 (5 x), reconstituted** | | |
| AsnRS | 26 µM | 5.2 µM |
| IleRS | 28 µM | 5.6 µM |
| AlaRS | 31.25 µM | 6.25 µM |
| PheRS1 | 50.5 µM | 10.1 µM |
| PheRS2 | 50.5 µM | 10.1 µM |
| MTF | 52.5 µM | 10.5 µM |
| EF-Ts | 75 µM | 15 µM |
| IF3 | 25 µM | 5 µM |
| IF1 | 84 µM | 16.8 µM |
| **LD3 (10 x)** | | |
| AspRS | 10 x | 1 x |
| ProRS | 10 x | 1 x |
| GlyRS1 | 10 x | 1 x |
| GlyRS2 | 10 x | 1 x |
| GluRS | 10 x | 1 x |
| RF3 | 10 x | 1 x |
| RF2 | 10 x | 1 x |
| RRF | 10 x | 1 x |
| EF-G | 10 x | 1 x |
| IF2 | 10 x | 1 x |
| **Enzyme mix (6 x)** | | |
| T7 RNAP | 120 ng/µL | 20 ng/µL |
| Myokinase | 30 ng/µL | 5 ng/µL |
| Creatine phosphokinase | 60 ng/µL | 10 ng/µL |
| Nucleoside-diphosphate kinase | 12 ng/µL | 2 ng/µL |
| RNAse Inhibitor | 1.5 U/µL | 0.25 U/µL |
| Inorganic pyrophosphatase | 6 U/µL | 1 U/µL |
| EF-Tu | 30 µM | 5 µM |
| Ribosomes | 10.8 µM | 1.8 µM |
| HEPES·KOH pH 8.0 | 50 mM | 8.3 mM |
| DTT | 6 mM | 1 mM |
| Glycerol | 2 % | 0.3 % |
| **Energy mix (4 x)** | | |
| Potassium Glutamate | 400 mM | 100 mM |
| Spermidine | 10mM | 2.5 mM |
| ATP | 8 mM | 2 mM |
| GTP | 8 mM | 2 mM |
| CTP | 4 mM | 1 mM |
| UTP | 4 mM | 1 mM |
| Sodium Creatine Phosphate | 80 mM | 20 mM |
| Folinic Acid | 40 mM | 10 mM |
| HEPES-KOH pH 7.5 | 200 mM | 50 mM |
| Mg(OAc)2 | 52 mM | 13 mM |
| DTT | 20 mM | 5 mM |
| tRNA | OD260 216 | OD260 54 |
| Amino Acid mix | 4 mM | 1 mM |

*Supplementary Table 5:* S*equence of MSRP-22 sense strand*

|  |
| --- |
| **MSRP-22** |
| TGGGTGGGACCCCTTTCGGGGTCCTGCTCAACTTCCTGTCGAGCTAATGCCATTTTTAATGTCTTTAGCGAGACGCTACCATGGCTATCGCTGTAGGTAGCCGGAATTCCATTCCTAGGGCTCCACCGAAAGGTGGGCGGGGCTTCGGCCCAGGGACCTCCCCCTAAAGAGAGGACCCGGGATTCTACCGGTTTGGTAACTAGCTGATTGGCTAGTTACCACCCCT |

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