A plug-and-play approach to modulating recombinant protein production in *Escherichia coli*

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Why *E. coli*?

Why *E. coli*?

- Genetics and biochemistry intensely studied
- Genetic tools widely available
- Short doubling time
- Downstream processes well-established
- Regulatory compliances easy to meet
- Microbe of choice for recombinant protein production

Challenges in recombinant protein production

Perhaps the most serious challenge in recombinant protein production is insoluble expression. Possible solutions include:

Purify inclusion bodies and refold proteins

Decrease concentration of inducer

Lower cultivation temperature

Decrease aeration/agitation

} i.e., lower transcription

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These strategies may be easy to implement at laboratory scale, but pose challenges at large scales

5'-UTR sequences impact gene expression

5'-UTR sequences are untranslated regions of mRNA present upstream of the start codon.

5'-UTR sequences can impact gene expression levels due to their effect on

- mRNA half-life
- translation efficiency

5'-UTR of *E. coli ompA* gene

Genes Dev 6:135–148 (1992)

5'-UTR sequences may be used to modulate gene expression without recourse to tinkering with cultivation parameters

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A library of standard 5'-UTR sequences would be useful for modulating gene expression in a plug-and-play fashion

Standardization and Synthetic Biology

Standardization is a cornerstone of Synthetic Biology. A plethora of standards have been described for DNA parts:

- NOMAD
- Biobricks
- Bgl Bricks
- ...

These standards allow facile cloning and assembly of DNA parts.

Construction of a library of standard 5'-UTR parts

5'-UTR sequences were identified from literature that have been reported to have an impact on mRNA half-life in *E. coli*.

Twelve native* and four synthetic† 5'-UTR sequences were selected.

Prefix and suffix sequences in accordance with the RFC10 Biobrick standard were appended to the sequences.

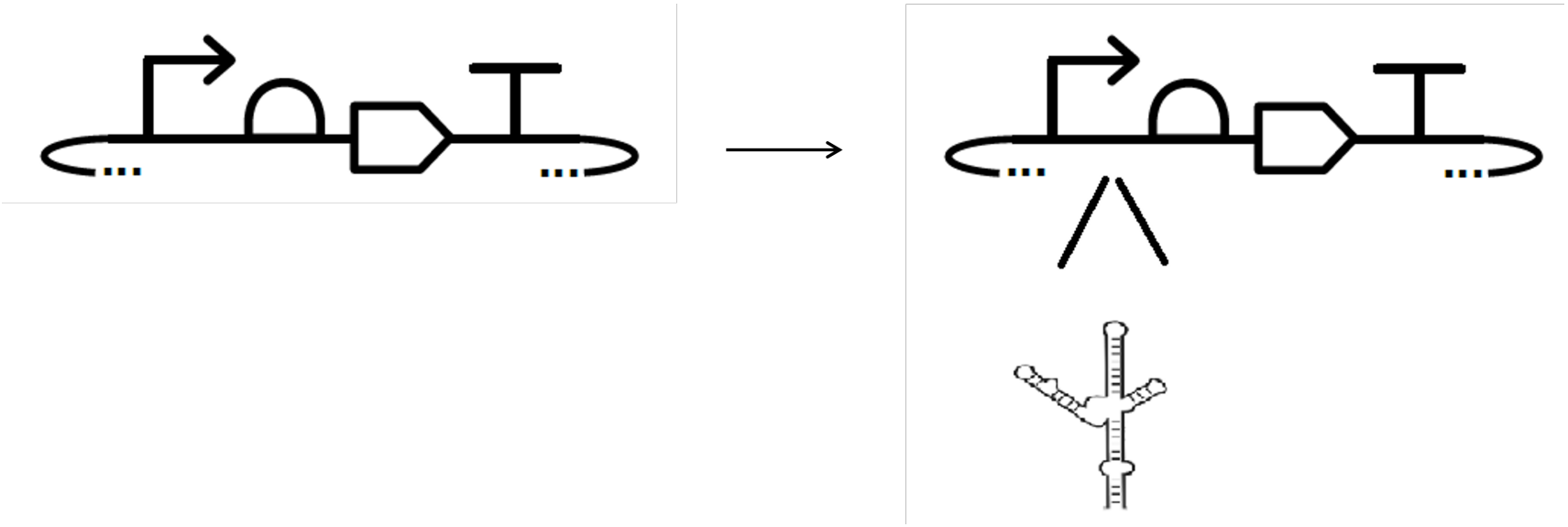
RFC10 standard was chosen as it is the most widely disseminated standard, thanks to the iGEM competition

* *Proc Natl Acad Sci USA* 101(9):2758–2763 (2004)
† *ACS Synth Biol* 7(9):2177–2188 (2018)

Gene	Function	mRNA half-life
B1016	hypothetical protein	13.6 min
katE	catalase; hydroperoxidase HPII(III)	17.9 min
yeiG	putative esterase (EC 3.1.1.-)	14.2 min
ynfA	hypothetical protein	16.3 min
ahpC	alkyl hydroperoxide reductase, C22 subunit; detoxification of hydroperoxides	18.4 min
yhjT	hypothetical protein	15.6 min

Gene	Function	mRNA half-life
wrbA	trp repressor binding protein; affects association of trp repressor and operator	22.7 min
sfsA	probable regulator of maltose metabolism	14.8 min
ompA	outer membrane protein	8.8 min
mopB	GroES, 10 KDa chaperone binds to Hsp60 in presence of Mg-ATP, suppressing its ATPase activity	6.9 min
cspF	cold shock protein	11.4 min
cspH	CspH cold shock-like protein	6.2 min

Gene	Function	mRNA half-life
2d	synthetic	12.9 min
2e	synthetic	7.5 min
2f	synthetic	7.5 min
2h	synthetic	9.3 min



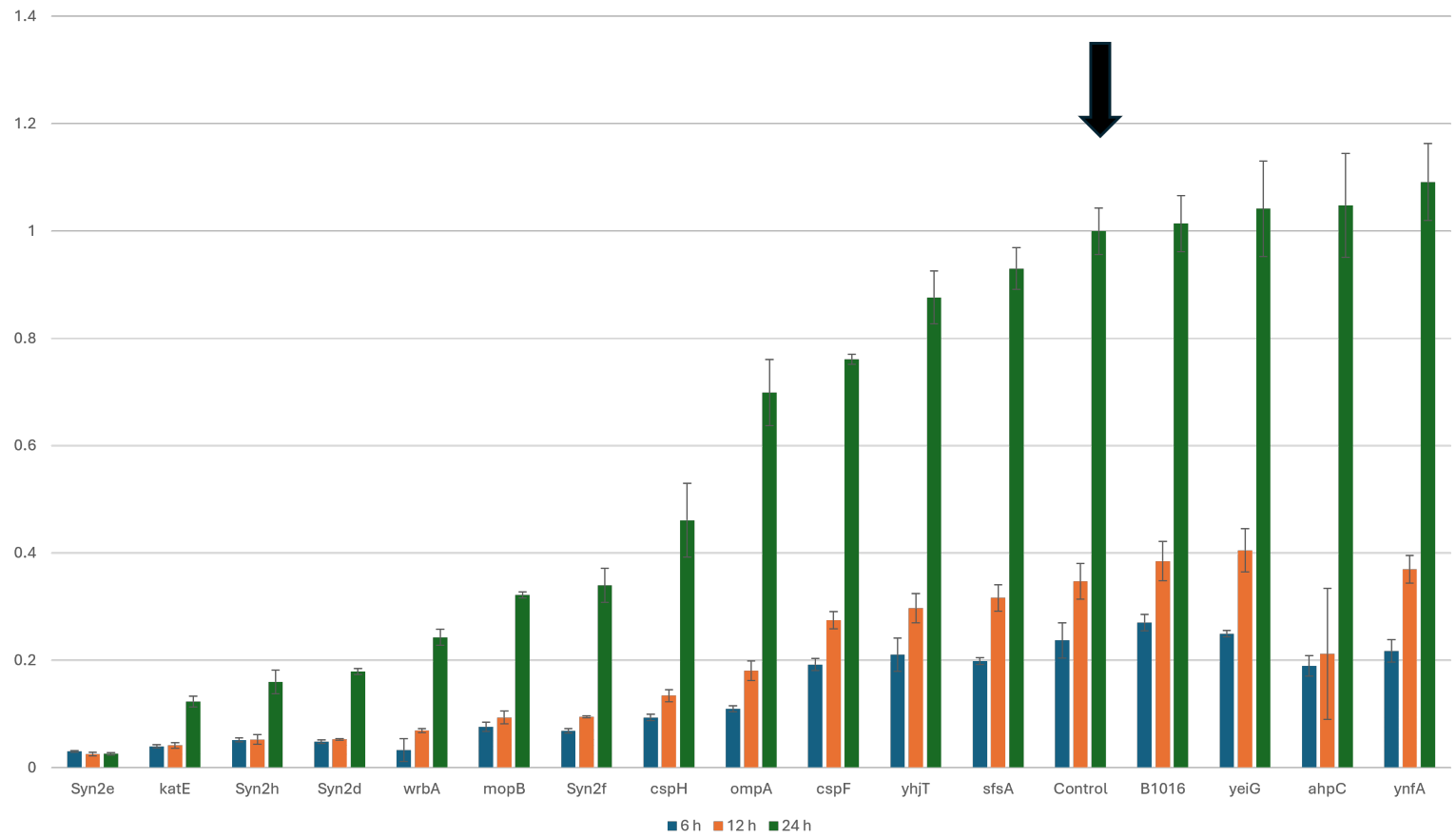
Biobricked[‡] 5'-UTRs were cloned upstream of the *rpf* gene in plasmid pSB1C3, and RFP fluorescence was compared with control lacking 5'-UTR in *E. coli* BL21(DE3)

[‡] BBrickIt! software: `https://synbiolab.net/bbrickit.html`

Impact of the 5'-UTR library

Normalized RFP fluorescence (FI/OD) ranged from 0.02-1.09 times that of the control.

This represents a 41-fold change in range of expression levels when the library of Biobricked 5'-UTR sequences was used.


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Syn2e
katE
Syn2h
Syn2d
wrbA
mopB
Syn2f
cspH
ompA
cspF
yhjT
sfsA
Control
B1016
yeiG
ahpC
ynfA
6 h
12 h
24 h

Construction of a 'petite' 5'-UTR library

The sizes of the 5'-UTR sequences used ranged from ∼10–100 bp.

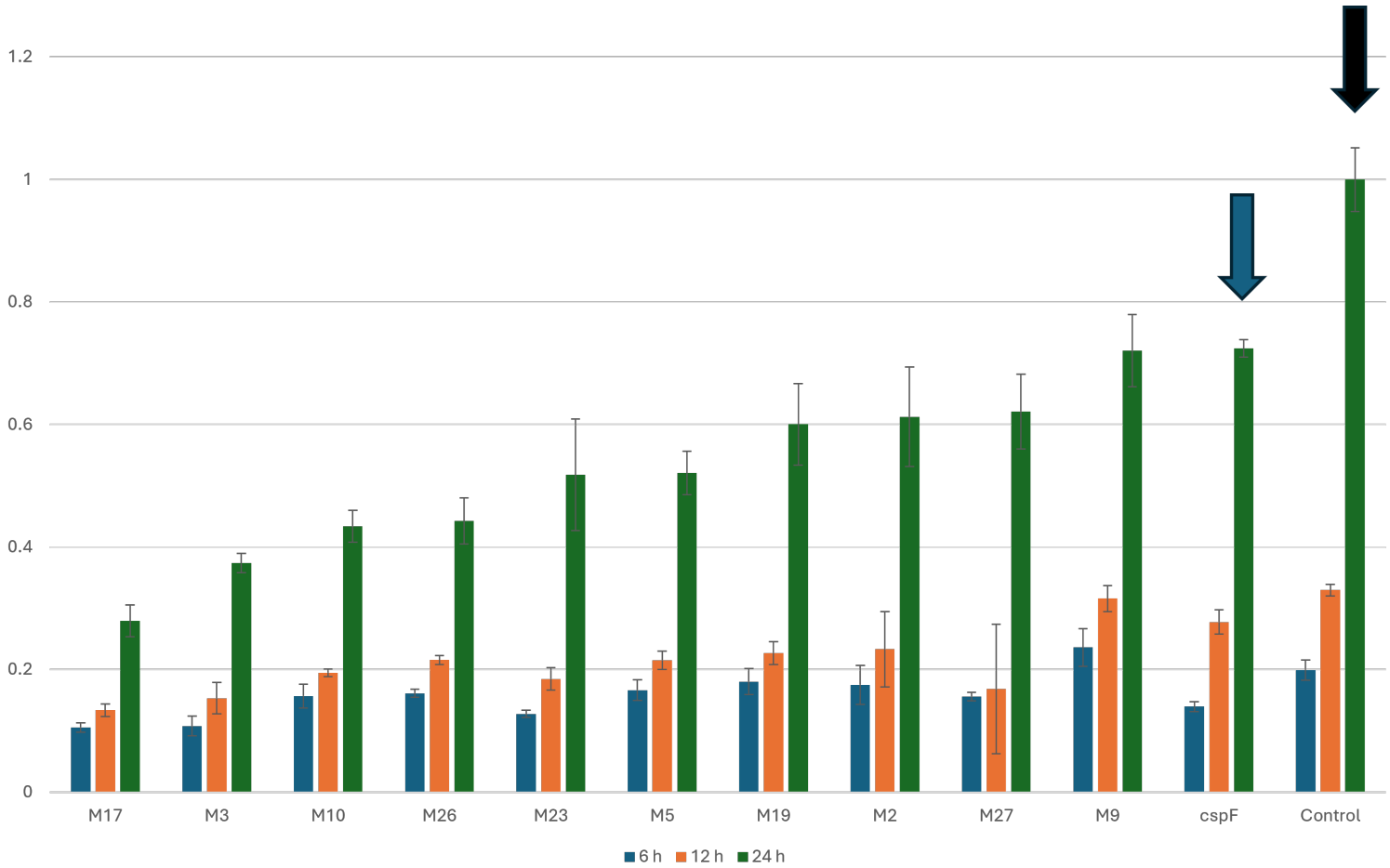
The smallest 5'-UTR sequence in the library was that present upstream of the *cspF* gene (9 bp).

Single point mutations were randomly generated in the 9 bp *cspF* 5'-UTR sequence *in silico*, and their impact on translation initiation rates were predicted.[§]

Ten mutants were selected to obtain evenly spaced predicted TIRs.

Mutant sequences were Biobricked and evaluated.

[§] *J Open Source Softw* 6(64):3362 (2021)



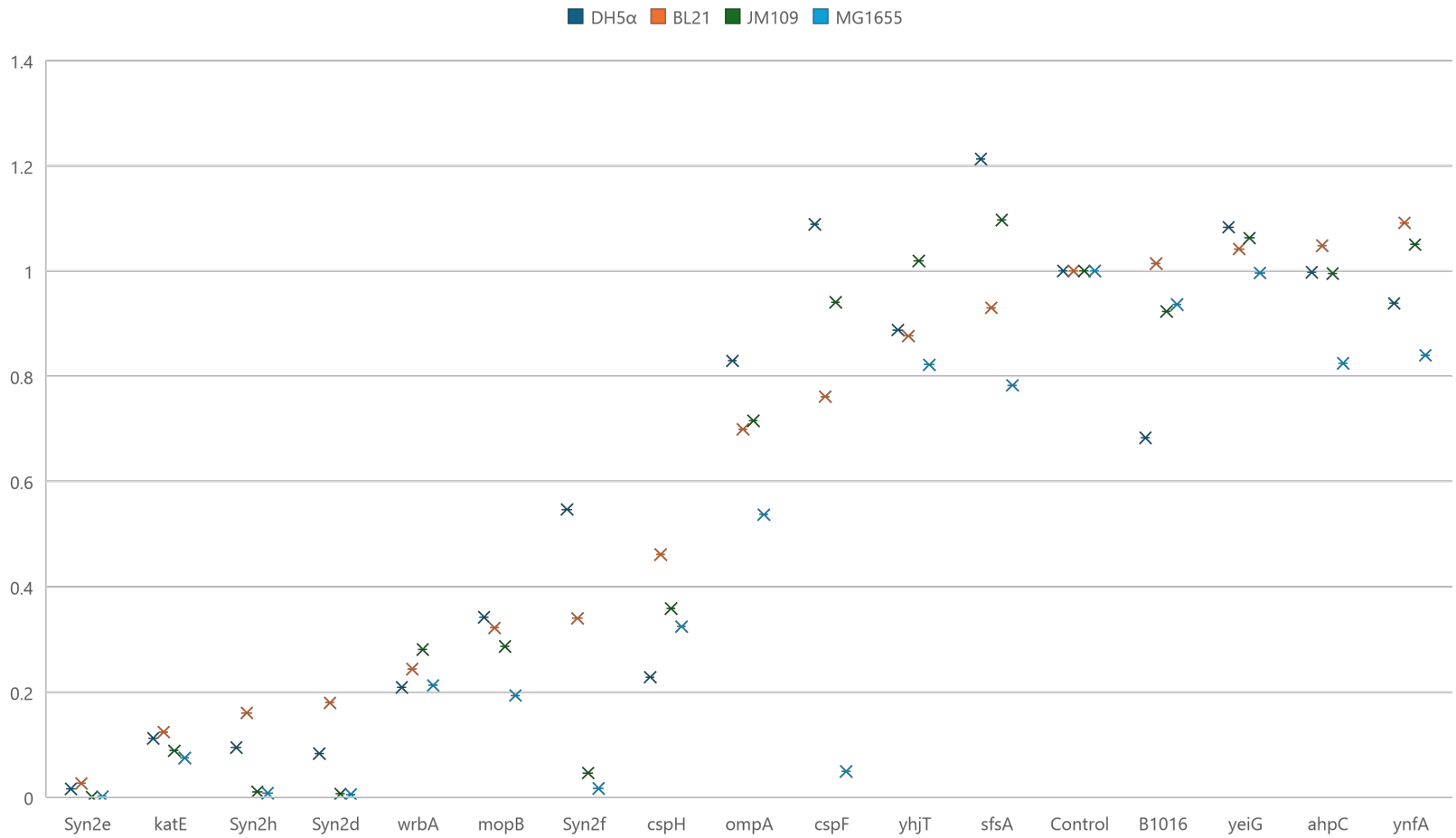
Normalized RFP fluorescence ranged from 0.27-0.72 times that of control

Impact of *E. coli* genetic background

The Biobricked 5'-UTR library was tested in other *E. coli* strains:

- DH5α
- JM109
- MG1655

Strains DH5α, JM109 and MG1655 belong to the K-12 lineage, while BL21(DE3) belongs to the B lineage

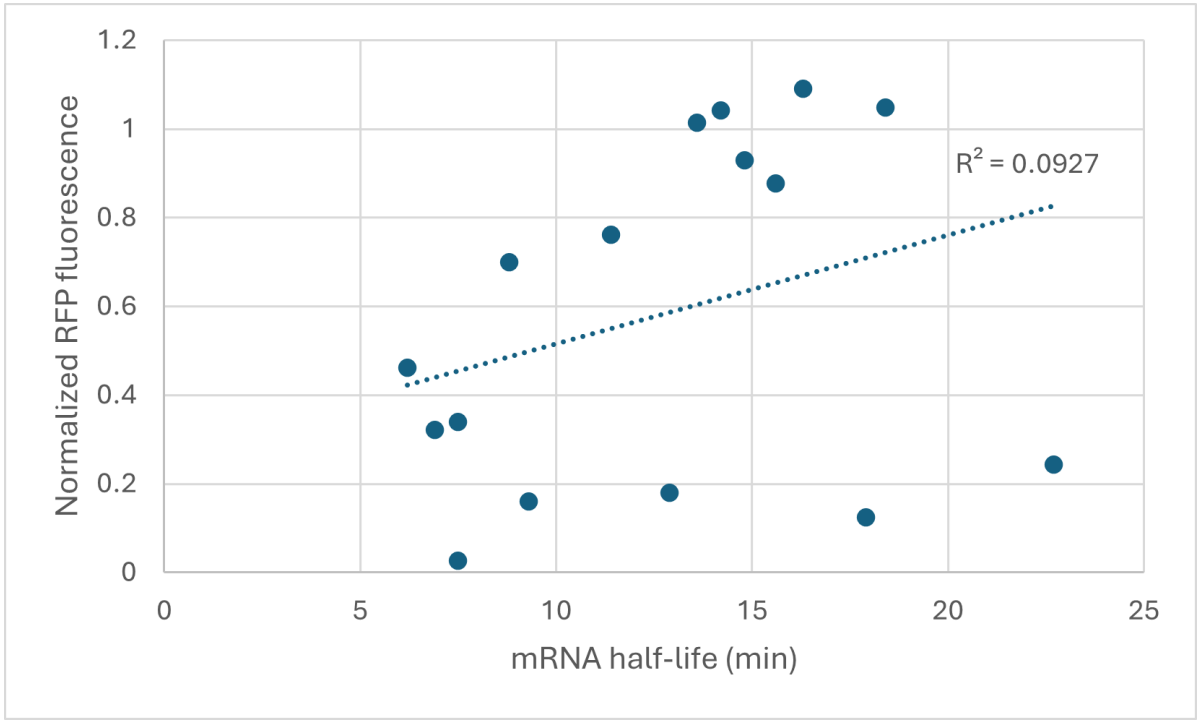

DH5α
BL21
JM109
MG1655
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Syn2e
katE
Syn2h
Syn2d
wrbA
mopB
Syn2f
cspH
ompA
cspF
yhjT
sfsA
Control
B1016
yeiG
ahpC
ynfA

	DH5α	**BL21**	**JM109**	**MG1655**
DH5α	1			
BL21	0.99	1		
JM109	0.98	0.99	1	
MG1655	0.97	0.97	0.96	1

Computed Spearman's coefficient of correlation for RFP expression under control of Biobricked 5'-UTR sequences in four *E. coli* strains

Impact of the 5'-UTR library is independent of *E. coli* strain genetic background

Interestingly, we found no correlation between the reported mRNA half-lives and normalized RFP fluorescence when RFP expression was modulated by the 5'-UTR sequences.


1.2
1
0.8
0.6
0.4
0.2
0
Normalized RFP fluorescence
0
5
10
15
20
25
mRNA half-life (min)
R² = 0.0927

Caveat emptor!

While mRNA and protein levels are usually weakly correlated, the coding sequence can be expected to impact mRNA half-life and protein expression, as the coding sequence cloned downstream of the 5'-UTR plays a crucial role in mRNA stability.

Conclusion

The Biobricked 5'-UTR library is a valuable addition to the Synthetic Biology toolkit.

The library allows modulation of gene expression in a plug-and-play fashion across *E. coli* strains.

Recombinant protein expression can be varied without recourse to tinkering with cultivation conditions

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