Design and evaluation of synthetic terminators for regulating mammalian cell transgene expression

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Supporting Information

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Accession codes of nucleotide sequences used in this manuscript

Human cytomegalovirus: M60321.1

Simian Virus 40: J02400.1

Human chromosomes, GRCh38.p2 assembly: NC_000006.12 (EEF1A1), NC_000007.14 (ACTB),

NC_000012.12 (GAPDH and RPL41), and NC_000017.11 (EIF4A1)

Equations

Because actinomycin D affects transcription of all RNA, any reference gene we chose would have had its own intrinsic degradation rate and thus fail to normalize the data. Instead, we calculated relative degradation by measuring the difference in degradation constants between our samples and the positive SV40 terminator control.

$$RNA_{1,t} = RNA_{1,t=0}e^{-\lambda_1 t}$$

$$RNA_{2,t} = RNA_{2,t=0}e^{-\lambda_2 t}$$

$$\frac{RNA_{2,t}}{RNA_{1,t}} = \frac{RNA_{2,t=0}}{RNA_{1,t=0}}e^{-(\lambda_2 - \lambda_1)t}$$

$$\ln \frac{RNA_{2,t}}{RNA_{1,t}} = -(\lambda_2 - \lambda_1)t + \ln \frac{RNA_{2,t=0}}{RNA_{1,t=0}}$$
(1)

This difference has an inverse linear relationship with steady state fluorescence levels, allowing us to determine whether the differences in GFP arise from differences in degradation rate.

$$\frac{d[mRNA]}{dt} = r_{promoter} - \lambda [mRNA]$$

$$\frac{d[GFP]}{dt} = k_{translation} [mRNA] - \delta_{GFP} [GFP]$$

$$[mRNA]_{S.S.} = \frac{r_{promoter}}{\lambda} = \frac{\delta_{GFP}}{k_{translation}} [GFP]_{S.S.}$$

$$\lambda_2 - \lambda_1 = r_{promoter} * \frac{k_{translation}}{\delta_{GFP}} \left[\frac{1}{[GFP]_2} - \frac{1}{[GFP]_1} \right] \quad (2)$$

Therefore, by assuming promoter expression (same promoter), GFP translation (same Kozak sequence), and GFP degradation (same peptide sequence) remain constant, we can determine if terminator sequences affect GFP by affecting degradation rate.

Supporting Tables and Graphics

Table S1: Terminators compared with the SV40 terminator based on hrGFP expression driven by *EIF4A1*-derived promoter in HT1080. Statistical significance determined by ANOVA and Tukey's HSD post-hoc testing. Expression difference corresponds to the difference of log mean hrGFP expression of the particular terminator relative to the SV40 terminator.

terminator pair	expression difference	Padjusted
HT1080.WT-f.SV40pA	-1.781	0.000
m.SV40pA.1-f.SV40pA	-0.312	0.000
m.SV40pA.2-f.SV40pA	-0.097	0.964
m.SV40pA.3-f.SV40pA	-0.078	0.999
m.SV40pA.4-f.SV40pA	-0.028	1.000
no.terminator-f.SV40pA	-1.187	0.000
T.ACTB.1-f.SV40pA	-0.363	0.000
T.ACTB.2-f.SV40pA	-0.812	0.000
T.ACTB.3-f.SV40pA	0.013	1.000
T.ACTB.4-f.SV40pA	0.042	1.000
T.ACTB.f-f.SV40pA	-0.387	0.000
T.EEF1A1.1-f.SV40pA	-0.411	0.000
T.EEF1A1.2-f.SV40pA	-0.252	0.000
T.EEF1A1.3-f.SV40pA	-0.537	0.000
T.EEF1A1.4-f.SV40pA	-0.432	0.000
T.GAPDH.1-f.SV40pA	-0.432	0.000
T.GAPDH.2-f.SV40pA	0.038	1.000
Tm.synth.01-f.SV40pA	-0.128	0.520
Tm.synth.02-f.SV40pA	-0.075	1.000
Tm.synth.03-f.SV40pA	-0.088	0.992
Tm.synth.04-f.SV40pA	-0.146	0.216
Tm.synth.05-f.SV40pA	-0.180	0.016
Tm.synth.06-f.SV40pA	-0.107	0.880
Tm.synth.07-f.SV40pA	-0.081	0.998
Tm.synth.08-f.SV40pA	-0.650	0.000
Tm.synth.09-f.SV40pA	-0.091	0.987
Tm.synth.10-f.SV40pA	-0.115	0.757
Tm.synth.11-f.SV40pA	-0.732	0.000
Tm.synth.12-f.SV40pA	-0.190	0.007
Tm.synth.13-f.SV40pA	-0.018	1.000
Tm.synth.14-f.SV40pA	-0.145	0.225
Tm.synth.15-f.SV40pA	-0.214	0.001
Tm.synth.16-f.SV40pA	-0.442	0.000
Tm.synth.17-f.SV40pA	-0.437	0.000
Tm.synth.18-f.SV40pA	-0.642	0.000
Tm.synth.19-f.SV40pA	-0.559	0.000
Tm.synth.20-f.SV40pA	-0.107	0.876
Tm.synth.21-f.SV40pA	-0.566	0.000
Tm.synth.22-f.SV40pA	-0.802	0.000
Tm.synth.23-f.SV40pA	-0.715	0.000
Tm.synth.24-f.SV40pA	-0.574	0.000
Tm.synth.25-f.SV40pA	-0.145	0.229
Tm.synth.26-f.SV40pA	-0.596	0.000
Tm.synth.27-f.SV40pA	-0.099	0.952
Tm.synth.28-f.SV40pA	-0.046	1.000

terminator pair	expression difference	Padjusted
Tm.synth.29-f.SV40pA	-0.494	0.000
Tm.synth.30-f.SV40pA	-0.444	0.000

Table S2: Terminators compared with the SV40 terminator based on hrGFP expression driven by *EIF4A1*-derived promoter with sub-population gating and an RFP transfection marker (constitutive mStrawberry expression driven by CMV promoter) in HT1080. Expression difference corresponds to the difference of log mean hrGFP expression of the particular terminator relative to the SV40 terminator.

terminator pair	expression difference	
no.terminator-f.SV40pA	-1.856	
T.GAPDH.1-f.SV40pA	-0.312	
T.GAPDH.2-f.SV40pA	0.059	
Tm.synth.02-f.SV40pA	-0.172	
Tm.synth.15-f.SV40pA	-0.188	
Tm.synth.17-f.SV40pA	-0.399	
Tm.synth.22-f.SV40pA	-1.017	

Table S3: Terminators compared with the SV40 terminator based on SEAP expression in HT1080. Statistical significance determined by ANOVA and Tukey's HSD posthoc testing. Expression difference corresponds to the difference in SEAP productivity of the particular terminator relative to SV40.

terminator pair	expression difference	Padjusted
no.terminator-f.SV40pA	-0.120	0.000
T.GAPDH.2-f.SV40pA	-0.057	0.000
T.ACTB.4-f.SV40pA	-0.020	0.011
Tm.synth.08-f.SV40pA	-0.112	0.000
Tm.synth.09-f.SV40pA	-0.079	0.000
Tm.synth.10-f.SV40pA	-0.109	0.000
Tm.synth.18-f.SV40pA	-0.100	0.000
Tm.synth.19-f.SV40pA	-0.014	0.384
Tm.synth.20-f.SV40pA	0.009	0.914
Tm.synth.23-f.SV40pA	-0.111	0.000
Tm.synth.24-f.SV40pA	-0.064	0.000
Tm.synth.25-f.SV40pA	-0.041	0.000
Tm.synth.26-f.SV40pA	-0.099	0.000
Tm.synth.27-f.SV40pA	-0.052	0.000
Tm.synth.28-f.SV40pA	-0.048	0.000

Table S4: Terminators compared with the SV40 terminator based on hrGFP expression driven by a strong promoter (pEEF1A1) in HT1080. Statistical significance determined by ANOVA and Tukey's HSD post-hoc testing. Expression difference corresponds to the difference of log mean hrGFP expression of the particular terminator relative to SV40.

terminator pair	expression difference	Padjusted
HT1080.WT-pEEF1A1.f.SV40pA	-2.443	0.000

-1.017	0.000
-0.395	0.000
-0.255	0.004
-0.197	0.084
-0.066	0.999
0.106	0.907
-0.228	0.019
0.022	1.000
0.107	0.901
-0.259	0.004
0.070	0.998
-0.042	1.000
-0.312	0.000
0.150	0.439
0.063	0.999
	-0.395 -0.255 -0.197 -0.066 0.106 -0.228 0.022 0.107 -0.259 0.070 -0.042 -0.312 0.150

Table S5: Terminators compared with the SV40 terminator based on hrGFP expression driven by *EIF4A1*-derived promoter in HEK293. Statistical significance determined by ANOVA and Tukey's HSD post-hoc testing. Expression difference corresponds to the difference of log mean hrGFP expression of the particular terminator relative to SV40.

terminator pair	expression difference	Padjusted
HEK293-f.SV40pA	-0.449	0.000
no.terminator-f.SV40pA	-0.301	0.000
T.GAPDH.2-f.SV40pA	0.073	0.183
T.ACTB.4-f.SV40pA	0.008	1.000
Tm.synth.08-f.SV40pA	-0.157	0.000
Tm.synth.09-f.SV40pA	-0.043	0.915
Tm.synth.10-f.SV40pA	-0.039	0.957
Tm.synth.18-f.SV40pA	-0.084	0.060
Tm.synth.19-f.SV40pA	0.046	0.857
Tm.synth.20-f.SV40pA	0.049	0.804
Tm.synth.23-f.SV40pA	-0.145	0.000
Tm.synth.24-f.SV40pA	0.031	0.996
Tm.synth.25-f.SV40pA	-0.086	0.049
Tm.synth.26-f.SV40pA	-0.116	0.001
Tm.synth.27-f.SV40pA	0.015	1.000
Tm.synth.28-f.SV40pA	-0.006	1.000

Table S6: Differential expression between a particular DSE and the DSE consensus from ANOVA analysis with Tukey's HSD post-hoc testing based on hrGFP expression driven by the pEIF4A1.636 promoter.

pairwise comparison	differential expression	Padjusted
Levitt-consensus	0.140	0.035
MC4R-consensus	0.220	0.002
CstF64.RRM-consensus	0.271	0.000
consensus+Levitt-consensus	0.570	0.000

Table S7: ANOVA analysis with Tukey's HSD post-hoc testing of the spacer 2, polyadenylation site, and DSE impact on hrGFP expression driven by the pEIF4A1.636 promoter.

Factor/interaction	p-value
spacer.2	0.033
PA.site	0.919
DSE	<2.20E-16
spacer.2:PA.site	9.96E-11
spacer.2:DSE	0.349
PA.site:DSE	0.779
spacer.2:PA.site:DSE	2.52E-10

Table S8: ANOVA analysis with Tukey's HSD post-hoc testing of the spacer 2, polyadenylation site, and DSE impact on SEAP expression driven by the pEIF4A1.636 promoter.

Factor/interaction	p-value
spacer.2	0.000
PA.site	0.755
DSE	3.351E-08
spacer.2:PA.site	4.94E-05
spacer.2:DSE	0.022
PA.site:DSE	0.503
spacer.2:PA.site:DSE	1.23E-03

Table S9: ANOVA analysis with Tukey's HSD post-hoc testing of the spacer 2, polyadenylation site, and DSE impact on hrGFP expression driven by the pEEF1A1.1356 promoter.

Factor/interaction	p-value
spacer.2	0.116
PA.site	0.539
DSE	1.08E-12
spacer.2:PA.site	0.527
spacer.2:DSE	0.035
PA.site:DSE	9.01E-05
spacer.2:PA.site:DSE	0.361

Table S10: Fully endogenous/synthetic genetic elements compared with viral-derived elements based on hrGFP expression in HT1080. rpCMV corresponds to reference CMV promoter, pEEF1A1 corresponds to the 1356-bp promoter derived from *EEF1A1*. Statistical significance determined by ANOVA and Tukey's HSD post-hoc testing. Expression difference corresponds to the difference of log mean hrGFP expression of the particular terminator relative to f.SV40pA.

terminator pair	expression difference	Padjusted
rpCMV.f.SV40pA-HT1080.WT	1.975	0.000

rpCMV.f.SV40pA-pEEF1A1.no.terminator	0.545	0.000
rpCMV.f.SV40pA-pEEF1A1.f.SV40pA	-0.471	0.000
rpCMV.f.SV40pA-pEEF1A1.T.GAPDH.2	-0.076	0.915
rpCMV.f.SV40pA-pEEF1A1.T.ACTB.4	-0.216	0.041
rpCMV.f.SV40pA-pEEF1A1.Tm.synth.13	-0.343	0.000

Table S11: Table of native/endogenous terminator sequences with putative spacer regions in lower case.

terminator	sequence 5' > 3'	length
f.SV40pA	CAGACATGATAAGATACATTGATGAGTTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAAT GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAccatttataagctgcAATAAAcaagttaacaacA AcaattgcattcatttTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCT CTACAAATGTGGTA	222
m.SV40pA.1	TGTAAccattataagctgcAATAAAcaagttaacaacAAcaattgcattcatttTATGTTTC	62
m.SV40pA.2	TGTAAccattataagctgcAATAAAcaagttaacaacAAcaattgcattcatttTATGTTTCAGGTTCAGGGGGAGGTGT GGGAGGTTTTTT	92
m.SV40pA.3	CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAAT GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAccatttataagctgcAATAAAcaagttaacaacA AcaattgcattcatttTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTT	192
m.SV40pA.4	TGTGATGCTATTGCTTTATTTGTAAccattataagetgcAATAAAcaagttaacaacAAcaattgcattcatttTATGTTTCAGGTTCAGGGGGGGGGGGGGGGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTA	142
T.EEF1A1.1	TGGTATtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTTTCTTTT	71
T.EEF1A1.2	TGGTATtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTTTCTTTTAACAGTTACTATGT TGGAATTGGTTACAAATTTTGGAGTGGATTTCAAAAGTGAGAGCTAACTTCAGTTGATTTCAAG GTAGTGCTTGGCTTTTTTTTGTTTA	173
T.EEF1A1.3	TGTGAAACCCAGTGTCTTAGACAACTGTGGCTTGAGCACCACCTGCTGGTATtcattacaaacttgctcacta cAATAAAtgaattttaagctttAAgatgaagtggcaTTTCTTTT	117
T.EEF1A1.4	TGTGAAACCCAGTGTCTTAGACAACTGTGGCTTGAGCACCACCTGCTGGTATtcattacaaacttgctcacta cAATAAAtgaattttaagctttAAgatgaagtggcaTTTCTTTTAACAGTTACTATGTTGGAATTGGTTACAAATT TTGGAGTGGATTTCAAAAGTGAGAGCTAACTTCAGTTGATTTCAAGGTAGTGCTTTGTTTT TGTTTA	219
T.GAPDH.1	TGTCATGTACcatcAATAAAgtaccctgtgctcaaccagtTActtgtcctgtcttattctagGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTC	99
T.GAPDH.2	TGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACcatcAATAAAgta ccctgtgctcaaccagtTActtgtcctgtcttattctagGGTCTGGGGCAGAGGGGAAGCTGGGCTTGTGTC	142
T.ACTB.f	GCGGACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAACTTGCGCAGAAAACAAG ATGAGATTGGCATGGCTTTATTTGTTTTTTTTTT	1065
T.ACTB.1	GCGGACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAACTTGCGCAGAAAACAAG ATGAGATTGGCATGGCTTTATTTGTTTTTTTTTT	700

terminator	sequence 5' > 3'	length
T.ACTB.2	GCGGACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAACTTGCGCAGAAAACAAG ATGAGATTGGCATGGCTTTATTTGTTTTTTTTTT	637
T.ACTB.3	TTGCTTTCGTGTAAATTATGTAATGCAAAATTTTTTTAATCTTCGCCTTAATACTTTTTTATTTTG TTTTATTTTGAATGATGAGCCTTCGTGCCCCCCCTTCCCCCCTTTTTTGTCCCCCAACTTGAGATGT ATGAAGGCTTTTGGTCTCCCTGGGAGTGGGTGGAGGCAGCCAGGGCTTACCTGTACactgacttgaga ccagttgAATAAAagtgcacaccttaaaaatGAggccaagtgtgacTTTGTGGTGTGGCTGGGTTGGGGGCAGCAGA GGGTGAACCCTGCAGGAGGGTGAACCCTGCAAAAGGGTGGGGCAGTGGGGGCCAAC	333
T.ACTB.4	TTGCTTTCGTGTAAATTATGTAATGCAAAATTTTTTTAATCTTCGCCTTAATACTTTTTTATTTTG TTTTATTTTGAATGATGAGCCTTCGTGCCCCCCCTTCCCCCCTTTTTTGTCCCCCAACTTGAGATGT ATGAAGGCTTTTGGTCTCCCTGGGAGTGGGTGGAGGCAGCCAGGGCTTACCTGTACactgacttgaga ccagttgAATAAAagtgcacaccttaaaaatGAggccaagtgtgacTTTGTGGTGTGGCTGGGTTGGGGG	270

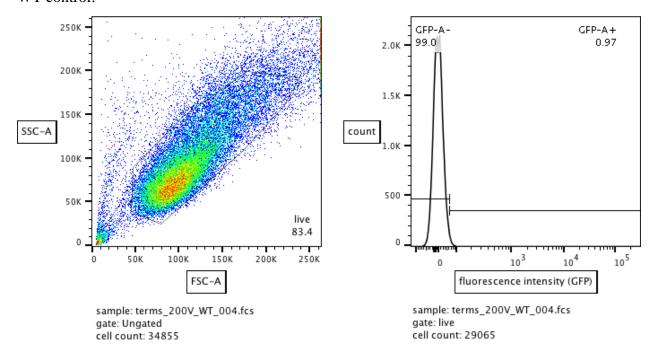
 $\textbf{Table S12:} \ \ \textbf{Table of synthetic terminator sequences}.$

terminator	sequence 5' > 3'	length
Tm.synth.1	TGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACcatcAATAAAgta ccctgtgctcaaccagtTActtgtcctgtcttattctagTGTGTTTT	113
Tm.synth.2	TGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACcatcAATAAAgta ccctgtgctcaaccagtTActtgtcctgtcttattctagTCTGTGTGTTTTTTTTTTTGTGTG	128
Tm.synth.3	TGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACcatcAATAAAgta ccctgtgctcaaccagtTActtgtcctgtcttattctagTGTGTTTTTCTGTGTGTTGTTTTTTTTTTTTTTTTTTT	136
Tm.synth.4	TGTAATGTAATGTAACatcAATAAAgtaccctgtgctcaaccagtTActtgtcctgtcttattctagGGTCTGGGGC AGAGGGGAGGCAAGCTGGGCTTGTGTC	109
Tm.synth.5	TGTAATGTAATGTAAAGTAAAAAgtaccctgtgctcaaccagtTActtgtcctgtcttattctagTGTGTTTT	80
Tm.synth.6	TGTAATGTAATGTAATGTAAcatcAATAAAgtaccctgtgctcaaccagtTActtgtcctgtcttattctagTCTGTGTTGGTTTTTTTTTGTGTG	95
Tm.synth.7	TGTAATGTAATGTAATGTAAcatcAATAAAgtaccctgtgctcaaccagtTActtgtcctgtcttattctagTGTTTTTCT GTGTTGTTTTTTTTTTTTTTTTTTTTTTT	103
Tm.synth.8	TGTAATGTAATGTAAAATAAAgtaccctgtgetcaaccagtTATGTGTTTT	56
Tm.synth.9	TGTAATGTAATGTAAAATAAAGtaccctgtgctcaaccagtTATCTGTGTGTTTTTTTGTGTG	71
Tm.synth.10	TGTAATGTAATGTAAAATAAAGtaccctgtgctcaaccagtTATGTGTTTTTCTGTGTGTTTTTTTGTGTGTG	79
Tm.synth.11	TGGTATtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTGTGTTTT	71
Tm.synth.12	TGGTATtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTCTGTGTGTTGTGTTTTTTGTGT	86
Tm.synth.13	TGGTATtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTGTGTTTTTCTGTGTGTTGTTTTTTTTTTGTGTG	94
Tm.synth.14	TGTAATGTAATGTAATGTAAtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTTTCTTTT AACAGTTACTATGTTGGAATTGGTTACAAAATTTTGGAGTGGATTTCAAAAGTGAGAGCTAACTT CAGTTGATTTCAAGGTAGTGCTTTGGCTTTTTTTGTTTA	187
Tm.synth.15	TGTAATGTAATGTAATGTAAtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTGTGTTT T	85
Tm.synth.16	TGTAATGTAATGTAATGTAAtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTCTGTGTGTTTGTGTGTGTGTGTGTGTGTGTGTGTGTGT	100
Tm.synth.17	TGTAATGTAATGTAATGTAAtcattacaaacttgctcactacAATAAAtgaattttaagctttAAgatgaagtggcaTGTGTTTTCTGTGTGTTTTTTTGTGTG	108
Tm.synth.18	TGTAATGTAATGTAAAATAAAtgaattttaagctttAATGTGTTTT	51
Tm.synth.19	TGTAATGTAATGTAAAATAAAtgaattttaagctttAATCTGTGTGTTTTTTTGTGTG	66
Tm.synth.20	TGTAATGTAATGTAAAGTAAAATAAAtgaattttaagctttAATGTGTTTTTCTGTGTGTTTTTTTGTG TG	74
Tm.synth.21	TGTAATGTAATGTAAATGTAAAATAAAagtgcacaccttaaaaatGATGTGTTTTTTCTGTGTGTTGTTTTTTTGTGTGTGT	77
Tm.synth.22	TGTAATGTAATGTAAAATAAAAagtgcacaccttaaaaatCATGTGTTTTTCTGTGTGTTGTTTTTT GTGTG	77

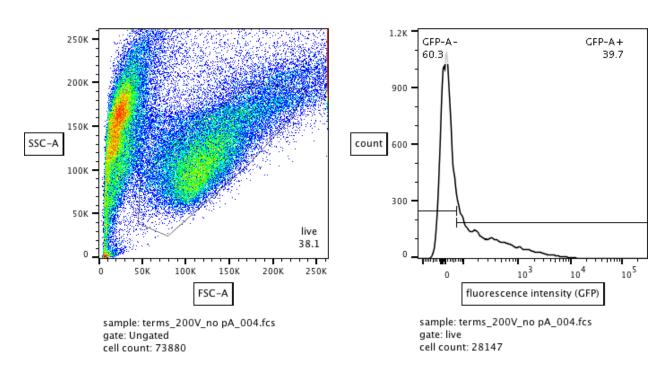
terminator	sequence 5' > 3'	length
Tm.synth.23	TGTAATGTAATGTAAAATAAAgtaccctgtgctcaaccagtCATGTGTTTT	56
Tm.synth.24	TGTAATGTAATGTAAAGTAAAATAAAgtaccctgtgctcaaccagtCATCTGTGTGTTGGTTTTTTGTGTG	71
Tm.synth.25	TGTAATGTAATGTAAAGTAAAATAAAgtaccetgtgctcaaccagtCATGTGTTTTTCTGTGTGTTGTTTTT TGTGTG	79
Tm.synth.26	TGTAATGTAATGTAAAATAAAtgaattttaagctttCATGTGTTTT	51
Tm.synth.27	TGTAATGTAATGTAAAATAAAtgaattttaagctttCATCTGTGTGTTTTTTTTTGTGTG	66
Tm.synth.28	TGTAATGTAATGTAAAGTAAAATAAAtgaattttaagctttCATGTGTTTTTCTGTGTGTTTTTTTTTGTG	74
Tm.synth.29	TGTAATGTAATGTAAAATAAAgtaccctgtgctcaaccagtCACGTGTTATTCATAAGCATT	67
Tm.synth.30	TGTAATGTAATGTAAAATAAAgtaccctgtgctcaaccagtCAGTTGTGTGTGTGTG	61

Figure S1: Representative cytometry plots with HT1080 cells from the same experiment.

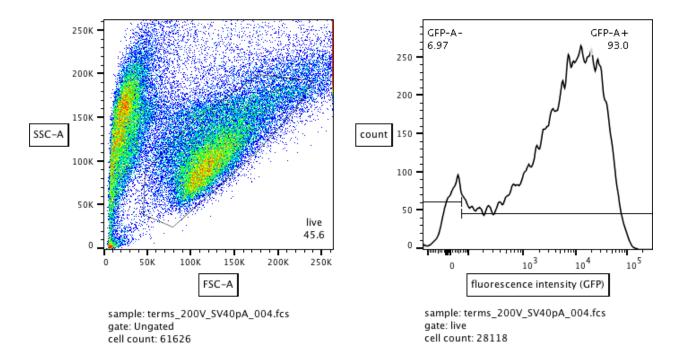
WT control:



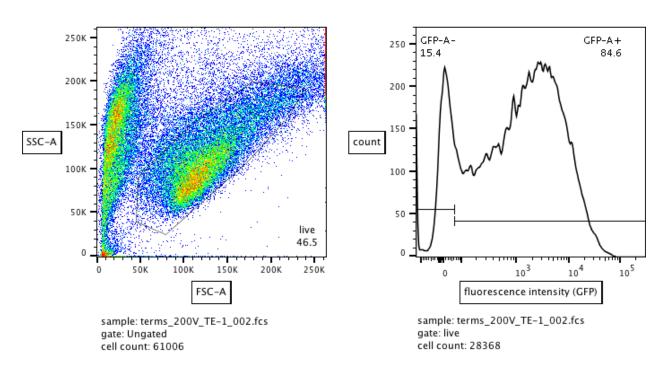
No terminator control:



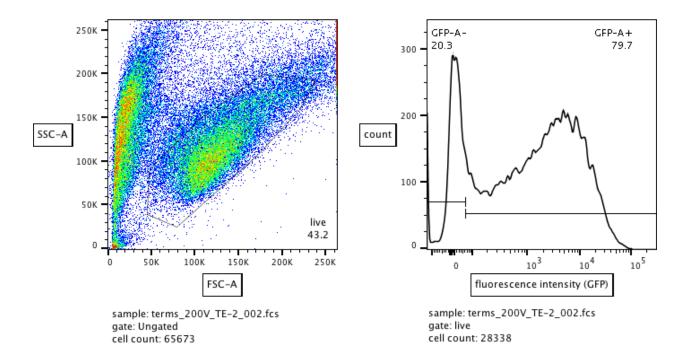
SV40pA reference control:



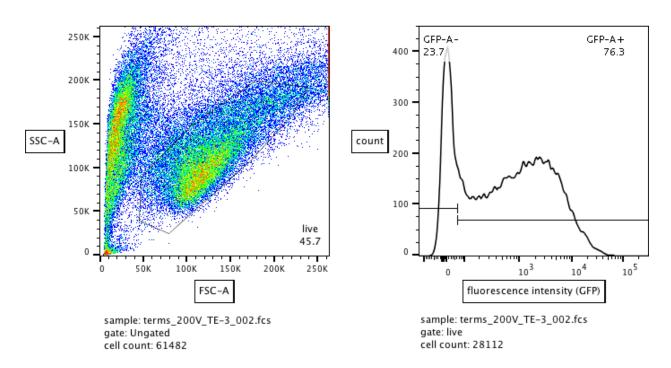
T.EEF1A1.1 variant:



T.EEF1A1.2 variant:



T.EEF1A1.3 variant:



T.EEF1A1.4 variant:

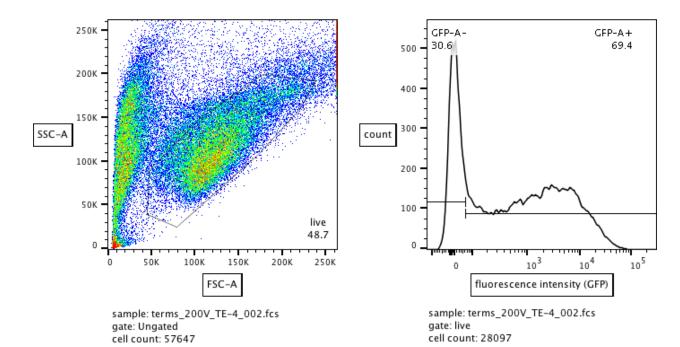
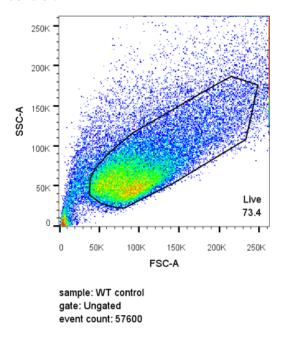
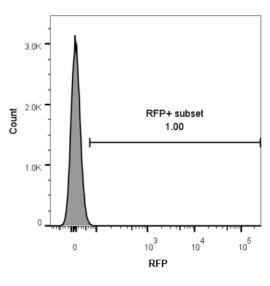


Figure S2: Representative cytometry plots with HT1080 cells from the same experiment, incorporating a strong constitutive mStrawberry (RFP) expression cassette as a transfection marker on the same plasmid as the hrGFP reporter.

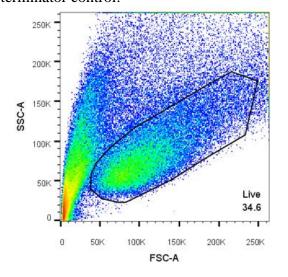
WT control:

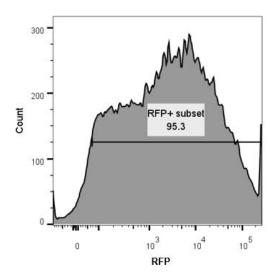




sample: WT control gate: Live event count: 42298

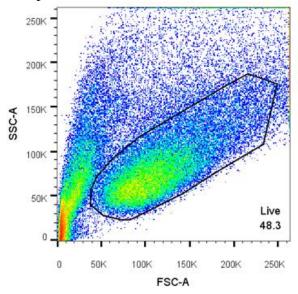
No terminator control:

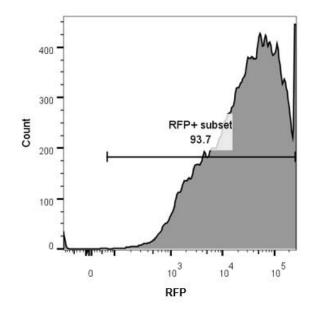




sample: No terminator control gate: Ungated event count: 124177 sample: No terminator control gate: Live event count: 42956

SV40pA reference control:





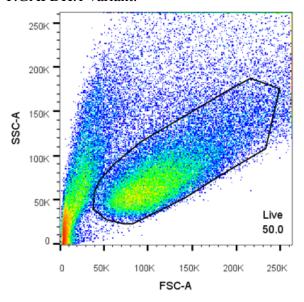
sample: SV40pA reference

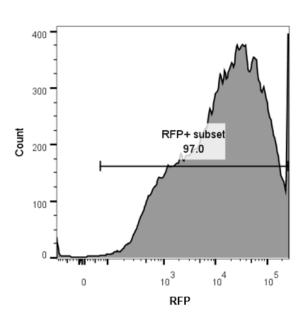
gate: Ungated event count: 86484

sample: SV40pA reference

gate: Live event count: 41811

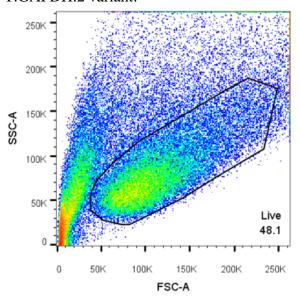
T.GAPDH.1 variant:

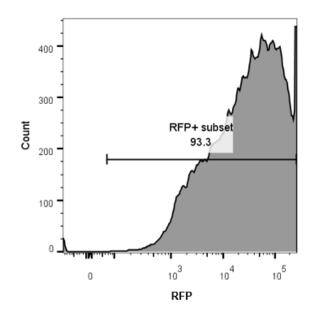




sample: T.GAPDH.1 gate: Ungated event count: 82730 sample: T.GAPDH.1 gate: Live event count: 41339

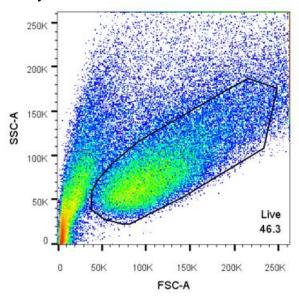
T.GAPDH.2 variant:





sample: T.GAPDH.2 gate: Ungated event count: 87542 sample: T.GAPDH.2 gate: Live event count: 42068

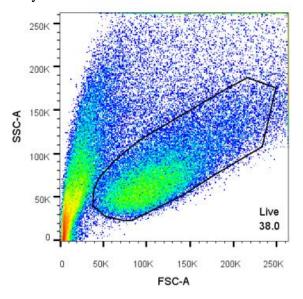
Tm.synth.02 variant:

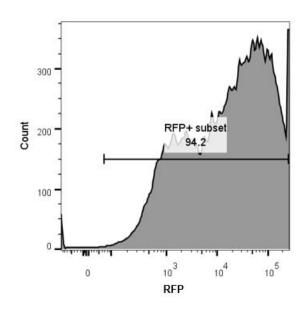


RFP+ subset 92.9 92.9 100 100 103 10⁴ 10⁵ RFP

sample : Tm.synth.02 gate: Ungated event count: 92371 sample: Tm.synth.02 gate: Live event count: 42809

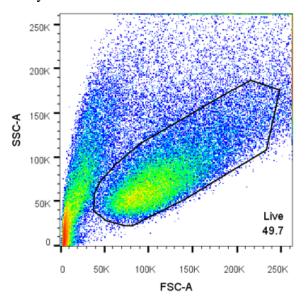
Tm.synth.15 variant:





sample: Tm.synth.15 gate: Ungated event count: 109145 sample: Tm.synth.15 gate: Live event count: 41489

Tm.synth.17 variant:



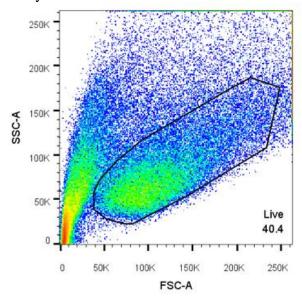
RFP+ subset 97.4

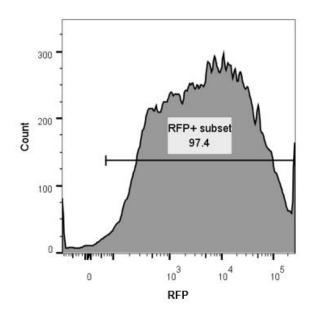
0 103 104 105

RFP

sample: Tm.synth.17 gate: Ungated event count: 83320 sample: Tm.synth.17 gate: Live event count: 41430

Tm.synth.22 variant:





sample: Tm.synth.22 gate: Ungated event count: 104142 sample: Tm.synth.22 gate:Live

event count: 42088