Supporting Online Material

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Table S1: The effect of the anti-Shine Dalgarno sequence on the ampicillin resistance.
The values depicted correspond to maximal ampicillin concentrations [g/L] at which growth was observed. The PciI recognition sequence is underlined, the minimal Shine-Dalgarno (SD) sequence in the wild type construct and the anti-SD sequence used are double-underlined, and the ATG start codon, of *bla* coding sequence, is typed in boldface. Numbers in brackets indicate the next ampicillin concentrations tested above the tolerated maximum. The ampicillin concentrations tested were ranging from 10 µg/mL to 2.5 g/mL.

Plasmid	Sequence 5′ → 3′	*m*-toluic acid concentrations	
		0 mM	0.1 mM
pAO-Tn			
wt	A<u>ACATGT</u>ACAATAATAAT<u>GGAG</u>TCATGAACAT**ATG**	0.010 (0.025)	0.10 (0.25)
anti-SD	..................<u>CCTC</u>.....	No growth	No growth

Table S2: Nucleotide sequence composition and the phenotype of *E. coli* clones harbouring plasmids with different Tr- and Tn-UTRs identified in pAO-Tr- and pAO-Tn-based libraries.
The values depicted correspond to maximal ampicillin concentrations [g/L] at which growth was observed. LV-2 is a *Pm* 5′-UTR variant identified by Berg et al. 2009. The PciI recognition sequence is underlined, the minimal Shine-Dalgarno sequences are double-underlined, and the ATG start codon is typed in boldface. Numbers in brackets indicate the next ampicillin concentrations tested above the tolerated maximum. The ampicillin concentrations tested were ranging from 10 µg/mL to 3 g/mL.

Plasmid	Sequence 5′ → 3′	*m*-toluic acid concentrations	
		0 mM	0.1 mM
pAO-Tr			
wt	AACATGT-ACAATAATAATGGAGTCATGAACAT**ATG**	0.025 (0.050)	0.25 (0.40)
LV-2	.......-..C......CA....T..........	0.025 (0.050)	1.0 (1.2)
r11	.......-..C.......C..................	0.015 (0.025)	0.60 (0.8)
r28	.......-.-T.........AA..........	0.015 (0.025)	0.80 (1.0)
r31	.......T..C..G.....................	0.025 (0.050)	1.0 (1.2)
r36	.......-....GT....C....A..........	0.025 (0.050)	1.0 (1.2)
r50	.......T..........C.....T........	0.025 (0.050)	1.0 (1.2)
pAO-Tn			
wt	AACATGTACAATAATAATGGAGTCATGAACAT**ATG**	0.010 (0.025)	0.10 (0.25)
n2	........GTT.....-.....T........	0.25 (0.50)	2.0 (2.5)
n3	.........T.A.C.....AA..........	0.25 (0.50)	2.5 (3.0)
n13	.......G..........C........	0.25 (0.50)	2.0 (2.5)
n15	..........C....G.....T........	0.25 (0.50)	2.0 (2.5)
n16	...........C.....A........	0.25 (0.50)	1.5 (2.0)
n17	.......G..........T........	0.25 (0.50)	2.0 (2.5)
n18	........A..A.G.....T........	0.25 (0.50)	2.0 (2.5)
n24	.........T.....TA.....C........	0.25 (0.50)	2.0 (2.5)
n15	.........C.....G.....T..........	0.25 (0.50)	1.5 (2.0)
n17	........A.C.........T........	0.25 (0.50)	2.0 (2.5)
n23	............G.....T........	0.25 (0.50)	2.0 (2.5)
n25	...........G.....A........	0.25 (0.50)	1.0 (1.5)
n35	........A.......TA.....C........	0.25 (0.50)	1.5 (2.0)
n39	........-.........T........	0.25 (0.50)	2.0 (2.5)
n41	........A..C..C..C.....T........	0.25 (0.50)	2.5 (3.0)
n42	........A.....CT.....A........	0.25 (0.50)	2.0 (2.5)
n44	.......G.........A.....C........	0.25 (0.50)	2.5 (3.0)
n47	........AT.A.C...A.....T..........	0.25 (0.50)	2.5 (3.0)
n48	.........T...T.....AG..T........	0.25 (0.50)	2.0 (2.5)
n52	........GT...GA.....T........	0.25 (0.50)	2.0 (2.5)
n58	..........T...C..A.....AT.......	0.25 (0.50)	2.5 (3.0)
n59	.........T..T.G.TA.....T........	0.25 (0.50)	2.5 (3.0)

Table S3: Calculated translation initiation rates of Tr- and Tn-UTRs in combination with *bla* coding sequence.

The translation initiation rates (TIR) were calculated using the reverse engineering function of the RBS-calculator (Salis et al. 2009).

UTR	TIR
wt	522.82
LV-2	2,371.77
r31	716.42
r36	625.94
r50	1,407.17
n24	4,872.99
n44	7,306.38
n47	5,331.95
n58	7,306.38

Table S4: Calculated translation initiation rates of Tn-dual UTRs in combination with *bla* and *mCherry* coding sequence.

dTn1-6 represent sequences of six Tn-UTR dualUTR elements with maximal TIRs for *bla* (dTn 1-3) or *mcherry* (dTn 4-6).

	TIR	
Tn-UTR	***bla***	***mCherry***
wt	598.4	2,308.6
n24	5,332.0	5,678.7
n44	7,994.5	25,075.0
n47	5,834.1	12,766.2
n58	3,461.4	4,743.2
dTn1/dTn4	349,161.5	856,820.0
dTn2/dTn5	418,029.8	819,114.1
dTn3/dTn6	478,456.9	655,630.7

Table S5: The sequence composition of the designed 5′-UTR DNA sequences.
The Shine-Dalgarno sequences are underlined, as specified by the RBS calculator, and the ATG start codon is typed in boldface. dTn1-6 represent sequences of six Tn-UTR dualUTR elements with maximal TIRs with *bla* (dTn 1-3) or *mCherry* (dTn 4-6) coding sequences.

Name	Sequence 5′→3′
dTn1	GAGCTCCATTATTATTGTATATGTGCATCAATTACTAAGGAGGTATACT**ATG**
dTn2	GAGCTCCATTATTATTGTATATGTGCATCACCCTTTAAGGAGGTTTACT**ATG**
dTn3	GAGCTCCATTATTATTGTATATGTACCGTACCCGTTAAGGAGGTTTTCT**ATG**
dTn4	GAGCTCCATTATTATTGTATATGTAACAAGGCAGAATAAGGAGGTTCAT**ATG**
dTn5	GAGCTCCATTATTATTGTATATGTGGATATACCCAGTAAGGAGGTACAT**ATG**
dTn6	GAGCTCCATTATTATTGTATATGTATATAAGGATTAGAGGAGGTAATAT**ATG**

References

Berg, L., Lale, R., Bakke, I., Burroughs, N. & Valla, S. (2009) The expression of recombinant genes in *Escherichia coli* can be strongly stimulated at the transcript production level by mutating the DNA-region corresponding to the 5′-untranslated part of mRNA. Microb Biotechnol.
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