

Supplemental Table 1. Plasmids used in this study.

Plasmid Name	Descriptive Name	Backbone	Enzymes	Insert construction /PCR description	5' UTR	5' UTR Sequence (Modification from WT indicated by capital letters or dashes to represent deletions)
pKR68	Tn7_ <i>lacZ</i>	pMP749	BamHI/ HindIII	PCR of <i>lacZ</i> from pEX-pigR:: <i>lacZ</i>	-	-
pKR74	Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ <i>lacZ</i>	pKR68	KpnI/ NotI	Overlap extension PCR. Both <i>pdpA</i> 5'UTR and <i>tul4</i> promoter amplified from LVS gDNA	<i>pdpA</i>	agttatgttctaattaagtagacaatgatagcagtaaaagat
pKR84	Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ mut1_ <i>lacZ</i>	pKR68	KpnI/ NotI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgttctaattaagtaCTcaatgatTgcagtaaaagat
pKR85	Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ mut2_ <i>lacZ</i>	pKR68	KpnI/ NotI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgtActGattaagtagacaatgatagcagtaaaagat
pKR98	Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ badSD- <i>lacZ</i>	pKR68	KpnI/ NotI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgttctCCTCCATCAacaatgatagcagtaaaagat
pKR99	Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ ideal_ movedSD- <i>lacZ</i>	pKR68	KpnI/ NotI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgAAGGAGGTCCATCAacaatgatagcagtaaaagat
pKR88	Mini_ Tn7_ R6Kg	pKR55	NspI	R6K λ origin from pKL91	-	
pKR89	Tn7_ <i>Ptul4</i> _ <i>tul4</i> UTR_ <i>lacZ</i> _ R6Kg	pKR88	BamHI/ KpnI/ NotI	PCR of <i>tul4</i> promoter and 5'UTR from LVS gDNA; <i>lacZ</i> amplified from pKR68; three-way ligation	<i>tul4</i>	gagtatatgtgaatatttaaaaaataggagatctatatgaaaaaaataattaag
pKR128	pYES2_ Tn7_ <i>lacZ</i>	pKR68	DraI/ PstI	<i>ura3</i> gene and 2 μ origin digested from pYES2	-	
pKR129	pYES2_ Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ idealSD- <i>lacZ</i>	pKR128	NotI/ KpnI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgttctAAGGAGGTCAacaatgatagcagtaaaagat
pKR130	pYES2_ Tn7_ <i>Ptul4</i> _ <i>pdp</i> AUTR_ <i>tul4</i> SD- <i>lacZ</i>	pKR128	NotI/ KpnI	PCR of <i>pdpA</i> 5'UTR from pKR74; modifications encoded on primers	<i>pdpA</i>	agttatgttctaAGGAGTATCacaatgatagcagtaaaagat
pKR145	pF- <i>tul4</i> UTR-GFP	pF	KpnI/ BamHI/ NotI	PCR of <i>tul4</i> promoter and 5'UTR from pKR89; PCR of <i>gfp</i> from IDT gBlock of super-folder GFP codon-optimized for LVS; three-way ligation	<i>tul4</i>	gagtatatgtgaatatttaaaaaataggagatctatatgaaaaaaataattaag
pKR146	pF- <i>pdp</i> AUTR-GFP	pF	KpnI/ BamHI/ NotI	PCR of <i>tul4</i> promoter and <i>pdpA</i> 5'UTR from pKR74; PCR of <i>gfp</i> from IDT gBlock of super-folder GFP codon-optimized for LVS; three-way ligation	<i>pdpA</i>	Agttatgttctaattaagtagacaatgatagcagtaaaagat
pKR151	pF- <i>mraY</i> UTR-GFP	pKR145	PacI/NotI	PCR of <i>mraY</i> 5'UTR from LVS gDNA	<i>mraY</i>	ataaaaaaattgaaccaattatttagacgctaatttgactctattaaaaaataacatctattataactccaaggcattaaacatttaaatatgctgattatctttt
pKR152	pF-FTL_0215 UTR-GFP	pKR145	PacI/NotI	PCR of FTL_0215 5'UTR from LVS gDNA	FTL_0215	ataattacaaaaataaaaaatctctagcctaaaaacattgttgaaaaattttataaaatatactgattgaaccttaagattt

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						tattttattatgaatatagttgactac
pKR156	pF- <i>mraY</i> UTR_mut1-GFP	pKR145	PacI/NotI	Overlap extension PCR of <i>mraY</i> 5'UTR from pKR151; modifications encoded on primers	<i>mraY</i>	ataaaaaatttgaaccaattatttagacgcta atTTtgactctattaaaCCCCGCCGatc tattataactccaaggtcattaaacattttaa atatatgctgatttatctttt
pKR157	pF- <i>mraY</i> UTR_mut2-GFP	pKR145	PacI/NotI	Overlap extension PCR of <i>mraY</i> 5'UTR from pKR151; modifications encoded on primers	<i>mraY</i>	ataaaaaatttgaaccaattatttagacgcta atTTtgactctattaaaaTATacatatctat tataatactccaaggtcattaaacattttaa atatgctgatttatctttt
pKR160	pF- <i>iglA</i> UTR-GFP	pKR151	KpnI/NotI	PCR of <i>iglA</i> 5'UTR from LVS gDNA	<i>iglA</i>	aagggtgttgtaaaaaaggacaataagat ggcaaaaaataaaatc
pKR161	pF-FTL_0222UTR-GFP	pKR151	PacI/NotI	PCR of FTL_0222 5'UTR from LVS gDNA	FTL_0222	aaaaataaaaaatttgtaatttagtcagacca ttaagtagaattttgagtaataatgtagatt taaattacacagaataattaaatttttaacc tgtagctcaagtcctca
pKR162	pF-FTL_0881 UTR-GFP	pKR151	PacI/NotI	PCR of FTL_0881 5'UTR from LVS gDNA	FTL_0881	aaaaattttactggataaaaaagtttcag atgTTTTtactatttagattttaacaaacata aaacaaataatagtaaataaggagaaaaa aatgaaaaagataattaca
pKR163	pF-FTL_1093 UTR-GFP	pKR151	PacI/NotI	PCR of FTL_1093 5'UTR from LVS gDNA	FTL_1093	ccttcaatttagactagatattgtagattagaa tatacttttctaagtttatatacattattaac aattatttaacaaataataaggtagtagatg ctaaaaactaagagc
pKR165	pF- <i>mraY</i> -mut5-UTR-GFP	pKR151	PacI/MfeI	PCR of portion of <i>mraY</i> 5'UTR from pKR151	<i>mraY</i>	_____ga cgctaattttgactctattaaaaaaataacata tctattataatactccaaggtcattaaacatttta aatatatgctgatttatctttt
pKR169	pF- <i>mraY</i> -idealSD-UTR-GFP	pKR151	PacI/NotI	PCR of <i>mraY</i> 5'UTR from pKR151; modifications encoded on primers.	<i>mraY</i>	ataaaaaatttgaaccaattatttagacgcta atTTtgactctattaaaaaaataacatatctatt ataactccaaggtcattaAGGAGGtta aatatatgctgatttatctttt
pKR172	pF- <i>hfq</i> UTR-GFP	pKR151	NotI/PacI	PCR of <i>hfq</i> 5'UTR from LVS gDNA	<i>hfq</i>	gaagttaattgttaaactgataaaataataa ctataacaaaataattaaaagggaagttagat aatgtcaagaatatcatct
pKR175	pF- <i>mraY</i> -mut6-UTR-GFP	pKR151	NotI/PacI	PCR of portion of <i>mraY</i> 5'UTR from pKR151	<i>mraY</i>	_____ataaaaaaata acatatctattataatactccaaggtcattaaa cattttaaatatatgctgatttatctttt

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pKR177	pF- <i>mraY</i> -mut7-UTR-GFP	pKR151	NotI/PacI	PCR of portion of <i>mraY</i> 5'UTR from pKR175; modifications encoded on primers	<i>mraY</i>	_____AGAattaaaaaata acatatctattataaactccaagggtcattaa cattttaaatatatgctgatttatctttt
pKR179	pF- <i>mraY</i> -mut8-UTR-GFP	pKR151	NotI/PacI	PCR of portion of <i>mraY</i> 5'UTR from pKR165; modifications encoded on primers	<i>mraY</i>	____GCCGGGGgactctattaaaaaat aacatatctattataaactccaagggtcattaa acattttaaatatatgctgatttatctttt
pKR180	pF- <i>mraY</i> -mut9-UTR-GFP	pKR151	NotI/PacI	PCR of portion of <i>mraY</i> 5'UTR from pKR165; modifications encoded on primers	<i>mraY</i>	_____AGTGAGattaaaaaat aacatatctattataaactccaagggtcattaa acattttaaatatatgctgatttatctttt
pKR182	pF- <i>mraY</i> -mut3-UTR-GFP	pKR151	NotI/PacI	PCR of <i>mraY</i> 5'UTR from LVS gDNA; modifications encoded on primers	<i>mraY</i>	ataaaaaattgaaccaattatttagacgcta attttgactctattaaaaaataacatatctatt ataatactccaCTACTattaaacattttaaa tatatgctgatttatctttt
pKR183	pF- <i>mraY</i> -mut4-UTR-GFP	pKR151	NotI/PacI	Overlap extension PCR of <i>mraY</i> 5'UTR from LVS gDNA; modifications encoded on primers	<i>mraY</i>	ataaaaaattgaaccaattatttagacgcta attttAGTGAGattaaaaaataacatatc tattataaactccaCTACTattaaacatttt aaatatatgctgatttatctttt
pF-nat	pF-nat	pF	PstI/NcoI	Nourseothricin-resistance cassette digested from pF3-MglA-V	-	-
pKR15	pF-nat- <i>rpsU2-V</i>	pF- <i>rpsU2-V</i> (Trautman n & Ramsey, 2022)	PstI/NcoI	Nourseothricin-resistance cassette digested from pF3-MglA-V	-	-
pKL111	pEX Δ <i>hfq</i>	pEX	BamHI/ KpnI/ NotI	PCR of regions flanking <i>hfq</i> from LVS gDNA; three-way ligation	-	-
pKR158	pEX- <i>hfq-V</i>	pKL02	KpnI/NotI	PCR of 3' end of <i>hfq</i> gene from LVS gDNA	-	-