**Supplemental Methods**

**A) Random sequence generation**

200 and 400 bp random sequences were generated using the RSAT random sequence generation web tool (http://rsat.sb-roscoff.fr/random-seq\_form.cgi) using a *C. elegans* specific Markov Model calibrated on non-coding upstream sequences with an oligonucleotide size of 6. The generated 200 bp sequences were screened for those that did not contain an ATG codon on the top strand and the first 4 sequences found were used. The 400 bp sequences were not screened for start codons.

**B) Description of plasmid constructs**

A list of plasmids, vectors, and oligonucleotides used in this study are found in Table S1 which includes references to all previously published plasmids, Addgene reference numbers (if available) and the sequence of all plasmids constructed for this study.

Plasmids NMp3055, NMp3358, NMp3467, NMp3468, NMp3469, NMp3643, NMp3689, NMp3694, NMp3695, NMp3698, NMp3700, NMp3702, NMp3703, NMp3704, NMp3706, NMp3724, NMp3725, NMp3727, NMp3751, NMp3767, NMp3770, NMp3774, NMp3777, NMp3821, and NMp3823 have been previously described in Nonet (2020). Plasmids NMp3760 and NMp3766 have been previously described in Dour and Nonet (2021). Plasmid NMp4045 has been previously described in (Nonet, 2021). Plasmids NMp3699, NMp3863, NMp3896, NMp3911, NMp3932, NMp3938, NMp3947, NMp3980, NMp3985, NMp4037, NMp4064, NMp4114, NMp4134, NMp4137, NMp4215, NMp4388, NMp4404, NMp4428, NMp4432, and NMp4441 have been previously described in Nonet (2023). NMp3460, NMp3795, NMp3837, NMp3844, NMp3851, NMp3854, NMp3855, NMp3899, NMp3909, NMp3928, NMp3979, NMp3981, NMp4002, NMp4096, NMp4099, NMp4100, NMp4145, NMp4232, NMp4233, NMp4341, and NMp4613 have been previously described in Knoebel et al. (2023). NMp4574 has been described in <<Nonet, 2024, #238800>>. pDD372 GFP-C1 has been previously described in Dickinson et al. (2018). pTA-attB and pTA-attP have been previously described in (Groth et al., 2000). RK1062 has been previously described in (Distel et al., 2009). NMp3795, NMp3969, NMp3970, NMp4065, NMp4066, NMp4366, NMp4367, NMp4368, NMp4370, NMp4371, NMp4624, NMp4625, NMp4626, NMp4627, NMp4734, NMp4735, NMp4736, NMp4846, NMp4857, and NMp4858 DNAs were synthesized by Twist Biosciences. The sequence of these DNAs is listed in Table S1.

NMp3771 DR274 5’ arm tetO 4X

tetO 4X was amplified from NMp3724 using NMo6575/6668, digested with EcoRI & HindIII, and inserted into similarly digested NMp3055 by ligation. The 7X tetO was truncated to 4X by PCR mediated internal priming within the repeat.

NMp3772 DR274 5’ arm tetO 2X

tetO 2X was amplified from NMp3724 using NMo6575/6668, digested with EcoRI & HindIII, and inserted into similarly digested NMp3055 by ligation. The 7X tetO was truncated to 2X by PCR mediated internal priming within the repeat.

NMp3797 DR274 5’ arm-CT QUAS 5X ∆pes-10

The QUAS 5X ∆pes-10 promoter from NMp3795 was inserted into NMp3698 using a BsaI GG reaction.

NMp3801 pLF3FShC QUAS 5X GFP

The QUAS 5X ∆pes-10 promoter from NMp3797, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3848 pLF3FShC2

The hybridized oligonucleotide pair NMo6788/6789 was inserted into SpeI & SbfI digested NMp3643 by ligation.

NMp3851 DR274 AAC TGG rps-13 3' UTR

The rps-13 3' UTR was amplified from N2 genomic DNA using NMo6806/6807 and inserted into NMp3844 using a BsaI GG reaction.

NMp3864 pLF3FShC2 rps-13t rab-3pl tetR-L-QF

The rps-13 3' UTR from NMp3851, rab-3 long promoter from NMp3837, tetR-L-QF from NMp3821, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp3848 using a SapI GG reaction.

NMp3905 DR274 5’ arm-CT phat-5p

The phat-5 promoter was amplified from NMp3358 using NMo6907/6908 and inserted into NMp3698 using a BsaI GG reaction.

NMp3907 DR274 SEC-3’ arm act-4 3’ FRT13r

The act-4 3’ UTR was amplified from NMp3864 using NMo6650/6906. This fragment and the hybridized oligonucleotide pair NMo6904/6905 were co-assembled into NMp3702 using a BsaI GG reaction.

NMp3917 pLF3FShC phat-5p tetR-L-QF act-4 3’ FRT13

The phat-5 promoter from NMp3905, tetR-L-QF from NMp3821, and the act-4 3’ UTR from NMp3907 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3947 DR274 5’ arm-CT nhx-2p

The nhx-2 promoter was amplified from N2 genomic DNA using NMo6972/6973 and inserted into NMp3698 using a BsaI GG reaction.

NMp3953 DR274 AAC TGG rps-12p hyg F24 rps13t F13r

The rps-12 promoter was amplified from N2 genomic DNA using NMo6886/6887. The rps-12 3' UTR was amplified from N2 genomic DNA using NMo6888/6889. These two fragments, along with the two hybridized oligonucleotide pairs NMo6968/6969 and NMo6884/6885, were co-assembled into NMp3844 using a BsaI GG reaction.

NMp3978 DR274 FP nls-phiC31

phiCN from NMp3969 and phiCC from NMp3970 were co-assembled into NMp3469 using a BsaI GG reaction.

NMp4004 pLF3FShC2 rps-12p F24 rps-13 F13 tetO 7X

The hybridized oligonucleotide pairs NMo7029/7030 and NMo6786/6787, along with the rps-12 F24 rps-13 integration site from NMp3953, the tetO 7X promoter from NMp3909, GFP-C1 from NMp3426, and the let-858 3’ UTR from NMp3979, were co-assembled into NMp3848 using a SapI GG reaction.

NMp4011 pLF3FShC tetO 2X GFP-C1

tetO 2X from NMp3772, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4012 pLF3FShC tetO 4X GFP-C1

tetO 4X from NMp3771, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4056 DR274 CT tetO 7X ∆mec-7

The hybridized oligonucleotide pair NMo7064/7065 was inserted into EcoRI & SphI digested NMp3899 by ligation.

NMp4058 pLF3FShC tetO 14X GFP-C1

tetO 7X from NMp3770, the tetO 7X mec-7 basal promoter from NMp4056, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4061 DR274 AAC TGG tetO 7X

tetO 7X was amplified from NMp4004 using NMo7073/7075 and inserted into NMp3844 using a BsaI GG reaction.

NMp4062 DR274 AAC CAA tetO 7X

tetO 7X was amplified from NMp4004 using NMo7073/7075 and inserted into NMp3855 using a BsaI GG reaction.

NMp4069 DR274 NT FLP sl2 mNG

FLP sl2 mNG was amplified from NMp3689 using NMo7046/7047, digested with SapI, and inserted into similarly digested NMp3980 by ligation.

NMp4071 pRMHEmB

attB from NMp4065 was digested with BsaI & SpeI and inserted into similarly digested NMp3643 by ligation.

NMp4072 pRMHEmP

attP from NMp4066 was digested with BsaI & SpeI and inserted into similarly digested NMp3643 by ligation.

NMp4073 pLF3FShC2 tetO 21X GFP-C1

tetO 7X from NMp4061, tetO 7X from NMp3770, the tetO 7X mec-7 basal promoter from NMp4056, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4075 pRMHEmB mec-4Sp tetR-L-QF

The mec-4 synthetic promoter from NMp3863, tetR-L-QF from NMp3821, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4071 using a SapI GG reaction.

NMp4077 DR274 5’ arm-CT tetO 1X ∆mec-7

The tetO 1X mec-7 basal promoter was amplified from NMp3899 using NMo7072/7077, DpnI digested, purified, kinased, and religated.

NMp4079 DR274 5’ arm-CT tetO 4X ∆mec-7

The tetO 4X mec-7 basal promoter was amplified from NMp3899 using NMo7072/7077, DpnI digested, purified, kinased, and religated.

NMp4080 pLF3FShC tetO 1X GFP-C1

The tetO 1X mec-7 basal promoter from NMp4077, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI Golden Gate (GG) reaction.

NMp4081 pLF3FShC mex-5p phiC31 sl2 FLP D5 sl2 mNG

The mex-5 promoter from NMp4037, phiC31 from NMp3978, gpd2/3 sl2 from NMp4002, FLP sl2 mNG from NMp4069, and the glh-2 3’ UTR from NMp3981 were co-assembled into NMp3643 using a SapI GG reaction.

NMp4082 pRMHEB

attB was amplified from NMp1668 using NMo7080/7081, digested with AflII & BsiWI, and inserted in similarly digested NMp4071 by ligation.

NMp4085 pRMHEP

attP was amplified from NMp1669 using NMo7082/7083, digested with AgeI & MfeI, and inserted into similarly digested NMp4072 by ligation.

NMp4086 pRMHEB mec-4Sp tetR-L-QF

The mec-4 synthetic promoter from NMp3863, tetR-L-QF from NMp3821, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4090 pRMHEB ehs-1p tetR-LL-QF act-4 3’

The ehs-1 promoter from NMp3896, tetR-LL-QF from NMp4045, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4091 pRMHEB nhx-2p tetR-LL-QF act-4 3’

The nhx-2 promoter from NMp3947, tetR-LL-QF from NMp4045, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4092 pRMHEB cup-4p tetR-LL-QF act-4 3’

The cup-4 promoter from NMp3938, tetR-LL-QF from NMp4045, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4093 pRMHEB myo-2p tetR-LL-QF act-4 3’

The myo-2 promoter from NMp3985, tetR-LL-QF from NMp4045, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4094 pRMHEB dpy-7p tetR-LL-QF act-4 3’

The dpy-7 promoter from NMp3932, tetR-LL-QF from NMp4045, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4101 pLF3FShC2 lexO 13X GFP-C1

lexO 5X from NMp4099, lexO 5X from NMp3703, the lexO 3X pes-10 basal promoter from NMp4096, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using a SapI GG reaction.

NMp4140 DR274 NT nls-scarlet-nls

nls-scarlet-nls was amplified from NMp3911 using NMo6476/7155 and inserted into NMp4134 using a BsaI GG reaction.

NMp4143 pRMHEP tetO 4X TIR1 V2A nls-scarlet

tetO 4X from NMp4079, TIR1 from NMp3460, V2A from NMp4114, nls-scarlet from NMp4140, and the tbb-2 3’ UTR from NMp3777 were co-assembled into NMp4085 using a SapI GG reaction.

NMp4146 DR274 CT ∆mec-7 noATG

The mec-7 basal promoter was amplified from NMp3767 to remove an 'ATG' codon in the far 5' of the basal promoter using NMo7177/7178, DpnI digested, purified, kinased, and religated.

NMp4161 pRMHEB vha-6p tetR-LL-QF

The vha-6 promoter was amplified from N2 genomic DNA using NMo7183/7184. This fragment, the tetR-LL-QF from NMp4045, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4082 using a SapI GG reaction.

NMp4173 pLF3FShC tetO 7X ∆pes-10 ∆mec-7 GFP-C1

The tetO 7X pes-10 basal promoter from NMp4145, the mec-7 basal promoter from NMp4146, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp4177-4200 DR274 5’ arm tetO 6X

Hybridized oligonucleotide pairs NMo7179/7182 and NMo7180/7181 were co-assembled into NMp3467 using a BsaI GG reaction. The oligos contain N so that each plasmids from 4177-4200 contains distinct sequences between each of the tetO sites.

NMp4201-4214 pLF3FShC tetO 6X GFP-C1

tetO 6X from NMp4177-NMp4200, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp4225 DR274 5’ arm UAS 3X

UAS 3X was amplified from NMp2507 (RK1062) using NMo7206/7207 and inserted into NMp3467 using a BsaI GG reaction.

NMp4236 pLF3FhC UAS 22X ∆pes-10 GFP-C1

UAS 11X from NMp3700, the UAS 11X pes-10 basal promoter rom NMp4232, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4237 pLF3FShC UAS 3X ∆pes-10 GFP-C1

UAS 5X from NMp4225, the pes-10 basal promoter from NMp3706, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4238 pLF3FShC UAS 14X ∆pes-10 GFP-C1

UAS 3X from NMp4225, the UAS 11X pes-10 basal promoter from NMp4232, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4251 pRMHEP tetO 4X GFP1-10 sl2 scarlet

tetO 4X from NMp4079, GFP1-10 from NMp4233, sl2 from NMp3928, scarlet from NMp3911, and the tbb-2 3’ UTR from NMp3777 were co-assembled into NMp4085 using an LguI GG reaction.

NMp4271 DR274 AAC TGG tetO 6X

tetO 6X was amplified from a mix of NMp4201-4214 using NMo7250/7252, purified, and inserted into NMp3844 using a BsaI GG reaction.

NMp4273 DR274 CAA TGG tetO 6X

tetO 6X was amplified from a mix of NMp4201-4214 using NMo7248/7249, purified, and inserted into NMp3854 using a BsaI GG reaction.

NMp4275 DR274 AAC CAA tetO 6X

tetO 6X was amplified from a mix of NMp4201-4214 using NMo7250/7251, purified, and inserted into NMp3855 using a BsaI GG reaction.

NMp4276 pLF3FShC2 tetO 12X GFP-C1

tetO 6X from NMp4271, tetO 6X from NMp4191, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using a SapI GG reaction.

NMp4277 pLF3FShC2 tetO 18X GFP-C1

tetO 6X from NMp4273, tetO 6X from NMp4275, tetO 6X from NMp4191, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using a SapI GG reaction.

NMp4314 DR274 SEC-3’ arm rps-2 3’

The rps-2 3’ UTR was amplified from N2 genomic DNA using NMo7282/7283 and inserted into NMp3702 using a BsaI GG reaction.

NMp4334 DR274 AAC CAA QUAS 5X

QUAS 5X was amplified from NMp3801 using NMo7312/7313 and inserted into NMp3855 using a BsaI GG reaction.

NMp4335 DR274 AAC TGG QUAS 5X

QUAS 5X was amplified from NMp3801 using NMo7312/7315 and inserted into NMp3844 using a BsaI GG reaction.

NMp4336 DR274 CAA TGG QUAS 5X

QUAS 5X was amplified from NMp3801 using NMo7314/7315, purified, and inserted into NMp3854 using a BsaI GG reaction.

NMp4337 DR274 CT lexO 8X ∆pes-10

The lexO 8X pes-10 basal promoter was amplified from NMp4100 using NMo6594/7307, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4345 DR274 CT UAS 3X ∆pes-10

The UAS 3X pes-10 basal promoter was amplified from NMp4237 using NMo6594/7207 and inserted into NMp3468 using a BsaI GG reaction.

NMp4355 pLF3FShCmP

A minimal attP site was amplified from NMp4072 using NMo6682/7329, purified, and digested with SalI & SbfI. The large insert from NMp3643 was similarly digested and inserted by ligation.

NMp4356 pLF3FShC UAS 6X GFP-C1

UAS 3X from NMp4225, the UAS 3X pes-10 basal promoter from NMp4345, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4357 pLF3FShC QUAS 10X GFP-C1

QUAS 5X from NMp3704, the QUAS 5X pes-10 basal promoter from NMp4341, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3643 using an LguI GG reaction.

NMp4358 pLF3FShC2 QUAS 15X GFP-C1

QUAS 5X from NMp3704, QUAS 5X from NMp4335, the QUAS 5X pes-10 basal promoter from NMp4341, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4359 pLF3FShC2 QUAS 20X GFP-C1

QUAS 5X from NMp4334, QUAS 5X from NMp4336, QUAS 5X from NMp3704, the QUAS 5X pes-10 basal promoter from NMp4341, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4364 pLF3FShC2 lexO 18X GFP-C1

lexO 5X from NMp4099, lexO 5X from NMp3703, the lexO 8X pes-10 basal promoter from NMp4337, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4377 DR274 5’ arm promRand1

A random 400bp AT-rich sequence from NMp4366 was inserted into NMp3467 using a BsaI GG reaction.

NMp4378 DR274 5’ arm promRand2

A random 400bp AT-rich sequence from NMp4367 was inserted into NMp3467 using a BsaI GG reaction.

NMp4379 DR274 5’ arm promRand3

A random 400bp AT-rich sequence from NMp4368 was inserted into NMp3467 using a BsaI GG reaction.

NMp4381 DR274 CT p200-1

A 200bp random AT-rich sequence from NMp4370 was inserted into NMp3468 using a BsaI GG reaction.

NMp4382 DR274 CT p200-2

Another 200bp random AT-rich sequence from NMp4370 was inserted into NMp3468 using a BsaI GG reaction.

NMp4383 DR274 CT p200-3

A third 200bp random AT-rich sequence from NMp4371 was inserted into NMp3468 using a BsaI GG reaction.

NMp4391 pLF3FShCmP tetO 7X p200-1 GFP-C1

tetO 7X from NMp3770, a 200bp AT-rich random 'promoter' from NMp4381, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4355 using an LguI GG reaction.

NMp4392 pLF3FShCmP tetO 7X p200-2 GFP-C1

tetO 7X from NMp3770, a 200bp AT-rich random 'promoter' from NMp4382, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4355 using an LguI GG reaction.

NMp4393 pLF3FShCmP tetO 7X p200-3 GFP-C1

tetO 7X from NMp3770, a 200bp AT-rich random 'promoter' from NMp4383, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4355 using an LguI GG reaction.

NMp4409 DR274 5’ PATC 900

A 900bp PATC-rich sequence from an F54A3 intron was amplified from N2 genomic DNA using NMo7352/7353 and inserted into NMp3467 using a BsaI GG reaction.

NMp4421 pLF3FShC2 tetO 7X 400-R1 ∆mec-7 GFP-C1

tetO 7X from NMp4061, the 400bp AT-rich random sequence from NMp4377, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4423 pLF3FShC2 tetO 7X 400-R3 ∆mec-7 GFP-C1

tetO 7X from NMp4061, the 400bp AT-rich random sequence from NMp4379, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4424 pLF3FShC2 tetO 7X 900 PATC ∆mec-7 GFP-C1

tetO 7X from NMp4061, a 900 bp PATC-rich sequence from NMp4409, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp3848 using an LguI GG reaction.

NMp4452 DR274 CT ∆act-4

The act-4 basal promoter was amplified from N2 genomic DNA using NMo7372/7373, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4453 DR274 CT ∆pals-5

The pals-5 basal promoter was amplified from N2 genomic DNA using NMo7374/7375, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4454 DR274 CT ∆tbb-2

The tbb-2 basal promoter was amplified from N2 genomic DNA using NMo7376/7377, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4476 DR274 CT ∆lys-8

The lys-8 basal promoter was amplified from N2 genomic DNA using NMo7392/7393 and inserted into NMp3468 using a BsaI GG reaction.

NMp4481 pHygG2r attPf

attP was amplified from NMp4085 using NMo7385/7386 and inserted into pHygG2r, which was amplified from NMp4404 using NMo7264/7265, using a BsaI GG reaction.

NMp4483 pHygRP3

attP from NMp4085 was digested with EcoRV & NotI and inserted into similarly digested NMp4428 by ligation.

NMp4484 pHygGP7

attP from NMp4085 was digested with EcoRV & NotI and inserted into similarly digested NMp4432 by ligation.

NMp4487 pHygR3 tetO 7X ∆act-4 GFP-C1

tetO 7X from NMp3770, the act-4 basal promoter from NMp4452, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4428 using an LguI GG reaction.

NMp4488 pHygR3 tetO 7X ∆pals-5 GFP-C1

tetO 7X from NMp3770, the pals-5 basal promoter from NMp4453, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4428 using an LguI GG reaction.

NMp4489 pHygR3 tetO 7X ∆tbb-2 GFP-C1

tetO 7X from NMp3770, the tbb-2 basal promoter from NMp4454, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4428 using an LguI GG reaction.

NMp4490 pHygR3 tetO 7X ∆lys-8 GFP-C1

tetO 7X from NMp3770, the lys-8 basal promoter from NMp4476, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4428 using an LguI GG reaction.

NMp4575 pHygGP6

The attP region from NMp4484 was digested with EcoO109I & SpeI and inserted into similarly digested NMp4481 by ligation.

NMp4579 pHygRP2

The attP region from NMp4483 was digested with SpeI & XhoI and inserted into similarly digested NMp4388 by ligation.

NMp4591 pHygGP16

The hybridized oligonucleotides NMo6788/6789 were inserted in AvrII & XhoI digested NMp4575 by ligation.

NMp4635 DR274 5’ arm tetOA 4X

tetOA 4X from NMp4624 was inserted into NMp3467 using a BsaI GG reaction.

NMp4636 DR274 5’ arm tetOB 4X

tetOB 4X from NMp4625 was inserted into NMp3467 using a BsaI GG reaction.

NMp4637 DR274 5’ arm tetOC 4X

tetOC 4X from NMp4626 was inserted into NMp3467 using a BsaI GG reaction.

NMp4638 DR274 5’ arm tetOD 4X

tetOD 4X from NMp4627 was inserted into NMp3467 using a BsaI GG reaction.

NMp4639 DR274 CAA TGG lexO 6X

lexO 6X from NMp4625 was inserted into NMp3854 using a BsaI GG reaction.

NMp4640 DR274 AAC CAA lexO 6X

lexO 6X from NMp4626 was inserted into NMp3855 using a BsaI GG reaction.

NMp4648 DR274 5’ arm lexO 6X

lexO 3X from NMp4624 and lexO 3X from NMp4627 were co-assembled into NMp3467 using a BsaI GG reaction.

NMp4651 pHygR2 tetOA 4X mNG

tetOA 4X from NMp4635, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4652 pHygR2 tetOD 4X mNG

tetOD 4X from NMp4638, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4657 pHygR2 tetOB 4X mNG

tetOB 4X from NMp4636, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4658 pHygR2 tetOC 4X mNG

tetOC 4X from NMp4637, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4669 pHygGP16 lexO 18X mNG

lexO 6X from NMp4640, lexO 6X from NMp4639, lexO 6X from NMp4648, the pes-10 basal promoter from NMp3706, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4591 using an LguI GG reaction.

NMp4674 pHygGP6 lexO 6X mNG

lexO 6X from NMp4648, the pes-10 basal promoter from NMp3706, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4713 DR274 CAA TGG 18sp

A 1.8kb PATC rich sequence was amplified from N2 genomic DNA using NMo7519/7520 and inserted into NMp3854 using a BsaI GG reaction.

NMp4714 DR274 5’ arm 13sp

A 1.3kb PATC rich sequence was amplified from N2 genomic DNA using NMo7521/7522 and inserted into NMp3467 using a BsaI GG reaction.

NMp4721 pHygG12 tetO 7X 04sp ∆mec-7 GFP tbb-2 3’

tetO 7X from NMp4061, the 400bp AT-rich random sequence from NMp4379, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4613 using an LguI GG reaction.

NMp4731 pHygG12 tetO 7X 13sp ∆mec-7 GFP tbb-2 3’

tetO 7X from NMp4061, a 1.3kb spacer from NMp4714, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4613 using an LguI GG reaction.

NMp4732 pHygG12 tetO 7X 18sp 13sp ∆mec-7 GFP tbb-2 3’

tetO 7X from NMp4062, a 1.8kb spacer from NMp4713, a 1.3kb spacer from NMp4714, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4613 using an LguI GG reaction.

NMp4733 pHygG12 tetO 7X 18sp 04sp ∆mec-7 GFP tbb-2 3’

tetO 7X from NMp4062, a 1.8kb spacer from NMp4713, the 400bp AT-rich random sequence from NMp4378, the mec-7 basal promoter from NMp3767, GFP-C1 from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4613 using an LguI GG reaction.

NMp4740 DR274 5’ arm tetO 13X

tetO 13X from NMp4734 was inserted into NMp3467 using a BsaI GG reaction.

NMp4741 DR274 5’ arm lexO 13X

lexO 13X from NMp4735was inserted into NMp3467 using a BsaI GG reaction.

NMp4742 DR274 5’ arm QUAS 13X

QUAS 13X from NMp4736 was inserted into NMp3467 using a BsaI GG reaction.

NMp4754 pHygGB6 lexO 13X mNG

lexO 13X from NMp4741, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4574 using an LguI GG reaction.

NMp4755 pHygGB6 QUAS 13X mNG

QUAS 13X from NMp4742, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4574 using an LguI GG reaction.

NMp4756 pHygR2 TGG GTA ∆mec-7 nls-GFP

The mec-7 basal promoter nls-GFP fragment was amplified from NMp4004 using NMo7567/7568, digested with XmaI, and inserted into MscI & XmaI digested NMp4651 by ligation.

NMp4765 DR274 SEC-3’ arm ter222

The tbb-2 3’ UTR was amplified from NMp4721 using NMo7573/7574. The rpl-2 3’ UTR was amplified from N2 genomic DNA using NMo7575/7576. The rps-2 3’ UTR was amplified from N2 genomic DNA using NMo7283/7577. All three fragments were co-assembled into NMp3702 using a BsaI GG reaction.

NMp4766 DR274 5’ arm nls-scarlet tbb-2 rev

The nls-scarlet tbb-2 3' UTR fragment was amplified from NMp4143 using NMo7578/7579 and inserted into NMp3467 using a BsaI GG reaction.

NMp4775 pHygR2 tbb-2 3' m7bp nls GFP

The tbb-2 3’ UTR from NMp3694 and a small double stranded (ds) oligonucleotide spacer made from hybridized oligos NMo7580/7581 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4777 DR274 TGG AAG promRand3

NMp4379 was amplified with NMo7582/7583, DpnI digested, purified, kinased, and religated.

NMp4782 pHygGP6 nls-scarlet tetO 7X nls-GFP

nls-scarlet from NMp4766, tetO 7X mec-7 basal promoter from NMp4056, nls-GFP from NMp4064, and the let-858 3’ UTR from NMp3766 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4797 DR274 SEC-3’ arm rps-0 3’

The rps-0 3’ UTR was amplified from N2 genomic DNA using NMo7592/7593 and inserted into NMp3702 using a BsaI GG reaction.

NMp4804 DR274 CT ∆act-4.2

An act-4 basal promoter fragment was amplified from NMp4487 using NMo7373/7612, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4805 DR274 CT ∆act-4.3

An act-4 basal promoter fragment was amplified from NMp4487 using NMo7373/7613, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4806 DR274 CT ∆act-4.4

An act-4 basal promoter fragment was amplified from NMp4487 using NMo7373/7614, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4807 DR274 CT ∆tbb-2.2

A tbb-2 basal promoter fragment was amplified from N2 genomic DNA using NMo7377/7616, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4808 DR274 CT ∆lys-8.2

A lys-8 basal promoter fragment was amplified from N2 genomic DNA using NMo7377/7615, purified, and inserted into NMp3468 using a BsaI GG reaction.

NMp4809 pHygR2 R400 unc-54 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the unc-54 3’ UTR from NMp3760 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4810 pHygR2 R400 act-4 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4811 pHygR2 R400 rps-2 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the rps-2 3’ UTR from NMp4314 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4812 pHygR2 R400 let-858 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the let-858 3’ UTR from NMp3766 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4825 pHygR2 R400 tbb-2 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4828 pHygR2 tetO 7X ∆act-4.2 GFP tbb-2 3’

tetO 7X from NMp3770, an act-4 basal promoter from NMp4804, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4829 pHygR2 tetO 7X ∆act-4.3 GFP tbb-2 3’

tetO 7X from NMp3770, an act-4 basal promoter from NMp4805, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4830 pHygR2 tetO 7X ∆act-4.4 GFP tbb-2 3’

tetO 7X from NMp3770, an act-4 basal promoter from NMp4806, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4831 pHygR2 tetO 7X ∆tbb-2.2 GFP tbb-2 3’

tetO 7X from NMp3770, a tbb-2 basal promoter from NMp4807, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4832 pHygR2 tetO 7X ∆lys-8.2 GFP tbb-2 3’

tetO 7X from NMp3770, a lys-8 basal promoter from NMp4808, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4833 pHygR2 tetO 7X ∆act-4 GFP let-858 3’

tetO 7X from NMp3770, the act-4 basal promoter from NMp4452, GFP from NMp3426, and the let-858 3’ UTR from NMp3766 were co-assembled into NMp4388 using an LguI GG reaction.

NMp4837 pHygG6 TGG GTA sl2 mNG glh-2 3'

The gpd2 mNG glh-2 3' UTR fragment was amplified from NMp4081 using NMo6565/7617, digested with SphI & XmaI, and inserted into similarly digested NMp4441 by ligation.

NMp4838 pHygG6 TGG GTA sl2 Scarlet tbb-2 3'

The gpd2 scarlet tbb-2 3' UTR fragment was amplified from NMp4251 using NMo6565/7617, digested with SphI & XmaI, and inserted into similarly digested NMp4441 by ligation.

NMp4839 pHygG6 tetO 7X ∆mec-7p Scarlet unc-54 sl2 mNG glh-2 3’

The tetO 7X mec-7 basal promoter from NMp3899, scarlet from NMp4137, and the unc-54 3’ UTR from NMp3760 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4840 pHygG6 tetO 7X ∆mec-7p Scarlet let-858 sl2 mNG glh-2 3’

The tetO 7X mec-7 basal promoter from NMp4899, scarlet from NMp4137, and the let-858 3’ UTR from NMp3766 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4841 pHygG6 tetO 7X ∆mec-7 scarlet tbb-2 3’ sl2 mNG glh-2 3’

The tetO 7X mec-7 basal promoter from NMp3899, scarlet from NMp4137, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4842 pHygG6 tetO 7X ∆pes-10p Scarlet unc-54 sl2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the unc-54 3’ UTR from NMp3760 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4843 pHygG6 tetO 7X p10bp Scarlet let-858 sl2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the let-858 3’ UTR from NMp3766 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4864 pHygR2 R400 QFAD m7bp nlsGFP

A random 400bp AT-rich spacer from NMp4777 and the QF activation domain amplified from NMp4045 using NMo7631/7632 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4867 DR274 5’ arm tetO 13-33

tetO 13X-33 from NMp4856 was inserted into NMp3467 using a BsaI GG reaction.

NMp4868 DR274 5’ arm tetO 13-37

tetO 13X-37 from NMp4857 was inserted into NMp3467 using a BsaI GG reaction.

NMp4869 DR274 5’ arm tetO 13-40

tetO 13X-40 from NMp4858 was inserted into NMp3467 using a BsaI GG reaction.

NMp4873 DR274 SEC-3’ arm ubl-1 3’

The ubl-1 3’ UTR was amplified from N2 genomic DNA using NMo7646/7647, purified, and inserted into NMp3702 using a BsaI GG reaction.

NMp4876 pHygGP6 tetO 13X-33 mNG

tetO 13X-33 from NMp4867, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4877 pHygGP6 tetO 13X-37 mNG

tetO 13X-37 from NMp4868, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4878 pHygGP6 tetO 13X-40 mNG

tetO 13X-40 from NMp4869, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4879 DR274 SEC-3’ arm rps-9 3’

The rps-9 3’ UTR was amplified from N2 genomic DNA using NMo7644/7645, purified, and inserted into NMp3702 using a BsaI GG reaction.

NMp4880 DR274 SEC-3’ arm eef-2 3’

The eef-2 3’ UTR was amplified from N2 genomic DNA using NMo7648/7649, purified, and inserted into NMp3702 using a BsaI GG reaction.

NMp4881 pHygGP6 tetO 13X-43 mNG

tetO 13X-43 from NMp4740, the mec-7 basal promoter from NMp3767, mNG from NMp3699, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4575 using an LguI GG reaction.

NMp4892 pHygR2 R400 rps-0 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the rps-0 3’ UTR from NMp4797 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4894 pHygR2 R400 glh-2 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the glh-2 3’ UTR from NMp4215 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4896 pHygR2 R400 rps-9 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the ubl-1 3’ UTR from NMp4873 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4898 pHygR2 R400 rps-9 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the rps-9 3’ UTR from NMp4879 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4899 pHygR2 R400 eef-2 3' m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the eef-2 3’ UTR from NMp4880 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4900 pHygR2 R400 ter222 m7bp nls-GFP

A random 400bp AT-rich spacer from NMp4777 and the ter-222 3’ UTR from NMp4765 were co-assembled into NMp4756 using an LguI GG reaction.

NMp4935 pHygG6 tetO 7X ∆pes-10p mNG glh-2 3' gpd2 scarlet tbb-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, mNG from NMp3699, and the glh-2 3’ UTR from NMp4215 were co-assembled into NMp4838 using an LguI GG reaction.

NMp4938 pHygG6 tetO 7X ∆pes-10p scarlet rps-0 3' sl2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the rps-0 3’ UTR from NMp4797 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4939 pHygG6 tetO 7X ∆pes-10p scarlet glh-2 3' sl2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the glh-2 3’ UTR from NMp4215 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4952 pHygG6 tetO 7X ∆pes-10 scarlet eef-2 3' gpd2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the eef-2 3’ UTR from NMp4880 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4953 pHygG6 tetO 7X ∆pes-10 scarlet act-4 3' gpd2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the act-4 3’ UTR from NMp3751 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4954 pHygG6 tetO 7X ∆pes-10 scarlet rps-9 3' gpd2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the rps-9 3’ UTR from NMp4879 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4955 pHygG6 tetO 7X ∆pes-10 scarlet ter222 gpd2 mNG glh-2 3’

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the ter-222 3’ UTR from NMp4765 were co-assembled into NMp4837 using an LguI GG reaction.

NMp4956 pHygG6 tetO 7X ∆pes-10 scarlet ubl-1 3' gpd2 mNG glh-2 3'

The tetO 7X pes-10 basal promoter from NMp3695, scarlet from NMp4137, and the ubl-1 3’ UTR from NMp4873 were co-assembled into NMp4837 using an LguI GG reaction.

NMp5030 DR274 5’ arm-CT tetO 7X Kozak

The tetO 7X Kozak 'promoter' was amplified from NMp3770 using NMo7772/7773, DpnI digested, purified, kinased, and religated.

NMp5031 DR274 5’ arm-CT tetO 7X ts Kozak

The tetO 7X ts Kozak 'promoter' was amplified from NMp3770 using NMo7772/7774, DpnI digested, purified, kinased, and religated.

NMp5034 pHygRP2 tetO 7X Kozak GFP tbb-2 3’

The tetO 7X Kozak 'promoter' from NMp5030, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4579 using an LguI GG reaction.

NMp5035 pHygRP2 tetO 7X ts Kozak GFP tbb-2 3’

The tetO 7X ts Kozak 'promoter' from NMp5031, GFP from NMp3426, and the tbb-2 3’ UTR from NMp3694 were co-assembled into NMp4579 using an LguI GG reaction.

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