# Supplemental Methods

# Overview of bipartite expression plasmid construction

Reporter constructs

I used the *∆pes-10* basal promoter (Thatcher et al., 2001), *GFP-C1* (Dickinson et al., 2018), and the *tbb-2* 3' UTR (Merritt et al., 2008) for initial reporter constructs (Figure S5). The *∆pes-10* promoter was also used in previous *C. elegans* bipartite reporter studies (Wei et al., 2012; Wang et al., 2017; Mao et al., 2019). Upstream activating sequences were either synthesized or subcloned from previous sources. Sequences described in Mao et al. (2019) were used for *tetO* 7X, sequences from a zebrafish expression vector (Schroeter et al., 2006) were used for *UAS* 11X, and synthetic oligonucleotides were used for *lexO* 5X and *QUAS* 5X. The *QUAS* 5X 96bp sequence is identical to that used by Wei et al. (2012) but spaced slightly differently relative to the *∆pes-10* promoter. The *lexO* 5X binding site sequence is based upon the Drosophila pJFRC18 8X *lexO* reporter (Pfeiffer et al., 2010), but contains only 5 copies of the identical binding site and spacer sequence. The *∆myo-2* (Okkema et al., 1993; Okkema and Fire, 1994), *∆hsp-16.1* (Hong et al., 2004), and *∆mec-7* (Duggan et al., 1998) were picked as alternate basal promoters because they appear to be the among the best characterized *C. elegans* promoters in the literature. The *act-4* 3' UTR was chosen because this gene is expressed in most, if not all, tissues in *C. elegans*, though there is considerable variation in expression levels as assayed by single cell RNA seq(Cao et al., 2017).

Driver constructs

To construct driver clones I used a modular system cloning the *mec-4* promoter, the DNA binding domains, the *QF* activating domain (*QFAD*), and the *tbb-2* 3’ UTR into SapI assembly vectors (Schwartz and Jorgensen, 2016; Dickinson et al., 2018). *QFAD* was chosen as the activation domain for all constructs because Mao et al. (2019) demonstrated it is more active than VP64. Some of the components were designed as codon optimized open reading frames with introns and synthesized, and others were amplified from previously existing functional clones or *C. elegans* wild type genomic DNA (see below and Table S3 for details). In brief, the DNA binding domains of *tetR* (Gossen and Bujard, 1992)*, lexA* (Riabinina et al., 2015) and *GAL4SK* (Wang et al., 2017) were codon optimized for *C. elegans*. *rtetR* (Mao et al., 2019) and *QF* (Subedi et al., 2014) were amplified from plasmids. *mec-4* promoter constructs driving *GAL4SK-QFAD*, *tetR-QFAD*, *rtetR-QFAD*, *QF*, and *lexA-QFAD* were assembled utilizing this method (Figure S6). Mao et al. (2019) used a tri-cistronic approach using the rtetR-*QFAD*::P2A::mKate::T2A::tTS for their studies of tet drivers. tTS is composed of a chimeric tetR DNA binding domain that does not dimerize with rtetR (Freundlieb et al., 1999), fused to a *pie-1* transcriptional repressor domain and was used to reduce the background observed in extrachromosomal arrays in the absence of doxycycline. With the exception of a *phat-5* promoter driven construct (Figure S8), all constructs I created contain only the driver, but no fluorescent protein marker or tTS repressor. I also assembled a construct with the *mec-4* promoter driving expression of the previously described *GAL4SK-VP64* driver (Wang et al., 2017). Among this first set of driver lines, only *GAL4SK-QFAD* drove detectable GFP expression when combined with appropriate reporter lines. I would not have described the non-functional driver clones and lines herein, except for the fact that integration of these clones constitutes the bulk of my insertion efficiency data.

Analysis of the non-functional *rtetR-QFAD* and *QF* designs, close derivatives of which have been shown to function in *C.* *elegans* (Wei et al., 2012; Mao et al., 2019), pointed to either a) the functionality of an intron incorporated into the synthetic *QFAD* or b) the lack of a flexible linker between the DNA binding domain and the activation domain. I re-engineered the vectors adding a flexible linker and shortening the QF activation domain to a.a. 650-816 as found in *lexA-QFAD* (Riabinina et al., 2015) (Figure S6). The *act-4* 3' UTR was also used in place of the *tbb-2* 3' UTR to facilitate the creating insertions that contain both a driver and a reporter without the insert plasmid containing sequence duplications. I assembled *tetR-L-QFAD*, *rtetR-L-QFAD*, *lexA-L-QFAD*, and *QF2* driver clones (Riabinina et al., 2015) with flexible linkers and a *QF* driver clone (Wei et al., 2012) from native sequences. *tetR-L-QFAD* was particularly difficult to create as I was unable to assemble ampicillin resistant integration clones containing the synthetic codon optimized *tetR*. Two modifications were made to assemble a clone: 1) stop codons were added to the intron, and 2) the C-terminal region of the synthetic *tetR* DNA binding domain was replaced with native sequences. A *QF2*-like clone is probably also used in Mao et al. (2019)but it is referred to as *QF* in the main publication (Figure 4A in Mao et al. 2019) and as *QF2* in their supplemental methods (clone TC690).

# Description of plasmid constructs

A list of plasmids used in this study are found in Table S3 which includes a reference to all previously published plasmids, Addgene reference #s (if available), and the sequence of all plasmids constructed for this study. Oligonucleotides are listed in Table S4.

# Landing site vectors

NMp3631 II loxP FRT FRT3 SEC. Chr II homology arms were amplified from *C. elegans* N2 genomic DNA using NMo6450/6451 and NMo6452/6453, an *FRT GFP his-58* fragment was amplified from genomic DNA of strain NM5195 using NMo6456/6457, the *rpl-28* promoter was amplified from N2 genomic DNA using NMo6454/6455, the SEC cassette was provided from pDD363, and *FRT3* as a double stranded (ds) oligonucleotide NMo6458/6469. The six inserts were co-assembled into NMp3421 in a SapI Golden Gate (GG) reaction.

NMp3632 FRT hygro sqt-1 Cre LoxP vector. The *hygR* gene was amplified from pDD363 using NMo6464/6465, a *sqt-1 hsp-16 Cre* fragment was amplified from pDD363 using NMo6466/6467, and *loxP* (NMo6472/6473) and *FRT* (NMo6463/6471) ds oligonucleotides were co-assembled into NMp3421 in a SapI GG Reaction.

NMp3649 pCFJ1272 FRT FRT3 landing. The *FRT FRT3* SEC cassette from NMp3631 was amplified using NMo6489/6490, digested with AvrII and SbfI and inserted into SpeI/PstI digested miniMos vector pCFJ1272. This construct contains a *loxP* flanked self-excision cassette (SEC) upstream of the *rpl-28* promoter which permits simple screening (*sqt-1* Rol phenotype) or selection (hygromycin B) for *miniMos* insertions and subsequent excision of the SEC (Dickinson et al., 2015). This leaves only a *loxP* site 5’ of the *rpl-28* promoter in the landing site.

NMp3689 pCFJ1272 mex-5 FLP FRT FRT3 landing. NMp3649 was amplified in two fragments using NMo6566/6567 and NMo6568/6569. The *mex-5* promoter *FLP sl2 mNeonGreen* cassette was amplified from genomic DNA of strain BN711 using NMo6570/6571 and the fragments were co-assembled in a SapI GG reaction. See NM3649 regarding SEC.

# FRT insert vectors

NMp3622 pBluescript FRT. pBluescript KS(+) was digested with PstI and XmaI and the ds oligonucleotide NMo6419/6420 was ligated into the vector.

NMp3623 pBluescript FRT3. pBluescript KS(+) was digested with PstI and XmaI and the ds oligonucleotide NMo6421/6422 was ligated into the vector.

NM3638 loxP MCS FRT3 FRT hygro Sqt Cre. The *hygR* gene was amplified from pDD363 using NMo6464/6465, a *sqt-1 hsp-16 Cre* fragment was amplified from pDD363 using NMo6466/6483, an MCS FRT3 fragment was amplified from NMp3623 using NMo6460/6461 and *loxP* (NMo6469/6473) and *FRT* (NMo6462/6463) ds oligonucleotides were co-assembled into NMp3421 in a SapI GG Reaction.

NM3643 pLF3FShC. NMp3638 was digested with SpeI and XmaI and the ds oligonucleotide NMo6485/6486 was ligated into the vector. This adds SapI sites to permit GG cloning into this vector.

# Insert containing vectors

NMp1147 pBluescript II SK (+) HpaI. pBluescript II SK(+) was modified to insert a HpaI site in the polylinker adjacent to ApaI using DpnI-mediated sited directed mutagenesis (Fisher and Pei, 1997) using oligonucleotides NMo1377 and NMo1378.

NMp2262 mec-7p Cherry gpd2 GFP-RAB-3. The *gpd2/3* trans-splice region was amplified from N2 genomic DNA using NMo3895/3896, *mCherry* was amplified from pCFJ104 using NMo3891/3892, the *elks-1* 3’ UTR was amplified from N2 genomic DNA genomic using NMo3893/3894. All 3 fragments were co-assembled into NcoI/NheI digested NMp1028 using an In-Fusion® reaction using the manufacturer’s protocol.

NMp2267 mec-7p Cherry V2A GFP-RAB-3. NMp2262 was digested with BamHI and NcoI and a double stranded(ds) oligonucleotide encoding V2A coding sequence (NMo3907/3908) was inserted by ligation.

NMp3055 DR274 U6. The *C. elegans* U6 promoter and the U6 terminator and sgRNA were amplified from p*U6::klp-12* sgRNA using NMo5074/5076 and NMo5075/

5077, respectively, then re-amplified as a single fragment using NMo5074/5075. The resulting product was digested with HindIII and inserted as a blunt/HindIII fragment into SwaI/HindIII digested DR274.

NMp3358 phat-5p Cherry V2A GFP-RAB-3. The *phat-5* promoter was amplified from N2 genomic DNA using NMo5845/5846, digested with PstI and NheI, and inserted into similarly digested NMp2267.

NMp3366 phat-5p Cherry V2A ANF-EMD. *ANF-EMD* was amplified from ‘pges-ANF-GFP’ (Table S3) using NMo5866/5867, digested with BspHI and EagI, and inserted into NcoI/EagI digested NMp3358. Sequencing of the resulting plasmid revealed that the *GFP* was actually *Emerald*.

NMp3410 pBS SAP II. NMp1147 was digested with XbaI and HindIII and a ds oligonucleotide NMo5946/5968 containing two SapI sites was inserted by ligation.

NMp3421 pSAP I. NMp3410 was amplified with NMo5992/5993, digested with EcoRI and re-ligated resulting in the removal of the third SapI site in NMp3410, and replacing it with an EcoRI site.

NMp3456 DR274 NT- BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6038/6039 was inserted by ligation.

NMp3467 DR274 5’arm-BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6057/6058 was inserted by ligation.

NMp3468 DR274 CT-BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6059/6060 was inserted by ligation.

NMp3469 DR274 FP-BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6061/6062 was inserted by ligation.

NMp3470 DR274 3’arm-BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6063/6064 was inserted by ligation.

NMp3591 DR274 CT-NT-BsaI. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide pair NMo6346/6347 was inserted by ligation. A design error limits the last base of the last amino acid before the NT ACG codon which must be G and the first base of the first codon of the CT clone after the GCG codon, which must be A. Use NMp3773 instead.

NMp3617 pSAP mec-4p GAL4SK-VP64. The *mec-4* promoter was amplified from N2 genomic DNA using NMo6384/6405, *GAL4SK-VP64* was amplified from genomic DNA of strain PS6963 using NMo6403/6404, and the *tbb-2* 3’UTR was amplified from pDD363 using NMo6406/6407. The three fragments were co-assembled into NMp3421 using a SapI GG reaction.

NMp3618 pSAP mec-4p GAL4SK-QFAD. The *mec-4* promoter was amplified from N2 genomic DNA using NMo6384/6405 and the *tbb-2* 3’UTR was amplified from pDD363 using NMo6406/6407. A codon optimized *GAL4SK*DNA binding domain gene fragment was synthesized (3610, Table S3) and a codon optimized *QF* activation domain gene fragment was synthesized (3611, Table S3). The four fragments were co-assembled into NMp3421 using a SapI GG reaction.

NMp3656 pLF3FShC phat-5p Cherry V2A GFP-RAB-3*.*  NMp3643 was digested with SbfI and MluI and a PstI/BssHII fragment containing *phat-5p cherry V2A GFP-rab-3* from NMp3366 was inserted.

NMp3657 pLF3FShC phat-5p Cherry V2A ANF-EMD. MMp3643 was digested with SbfI and MluI and a PstI/BssHII fragment containing the *phat-5p cherry V2A ANF-EMD* from NMp3358 was inserted.

NMp3658 pSAP scl-6 Emerald. The promoter and signal sequence of *scl-6* amplified from N2 genomic DNA using NMo6509/6510, *Emerald* amplified from NMp3366 using NMo6495/6496, and the coding and 3’ UTR of *scl-6* amplified from N2 genomic DNA using NMo6511/65112 were co-assembled into NMp3421 using a SapI GG reaction. This plasmid was created by Kelly Ma.

NMp3663 pSAP lys-8 Cherry. The promoter and signal sequence of *lys-8* amplified from N2 genomic DNA using NMo6519/6520, *cherry* amplified from NMp3366 using NMo6497/6498, and the coding and 3’ UTR of *lys-8* amplified from N2 genomic DNA using NMo6521/65122 were co-assembled into NMp3421 using a SapI GG reaction. This plasmid was created by Kelly Ma.

NMp3666 pCFJ1272 sqt cre Sap. NMp3649 was amplified with NMo6528/6529, digested with XmaI and re-ligated removing the *rpl-28p FRT GFP-His58* cassette from the plasmid.

NMp3671 tetOp cherry V2A GFP-rab-3.The *tetO* 7X *∆pes-10* promoter was amplified from TC358 using NMo6552/6553, digested with NheI and PstI and inserted into similarly digested NMp3358.

NMp3672 phat-5p rtetT-QFAD. The *rtetR-QFAD::P2A::mKate::T2A::tetR-pie1*

fragment from TC374 was amplified with NMo6548/6549 and digested with PstI and XbaI. The *phat-5* promoter was amplified from NMp3358 with NMo3794/6550, digested with PstI and XbaI and inserted by ligation.

NMp3673 Y8a9a.2p rtetR-QFAD. The *rtetR-QFAD::P2A::mKate::T2A::tetR-pie1* fragment from TC374 was amplified with NMo6548/6549 and digested with PstI and XbaI. The *Y8A9A.2* promoter was amplified from NMp3368 with NMo3794/6551, digested with PstI and XbaI and inserted by ligation.

NM3674 DR274 FP mCherry. *mCherry* was amplified from NMp2267 using NMo6546/6547 and inserted into NMp3469 using a BsaI GG reaction. This plasmid was created by Scott Dour.

NMp3681 pCFJ1272 Sqt Cre phat-5p rtetR-QFAD. NMp3666 was digested with SbfI and SpeI and the *phat-5p rtetR-QFAD::P2A::mKate::T2A::tetR-pie1* PstI/SpeI fragment from 3672 was inserted by ligation.

NMp3684 pLF3FShC tetO 7X mCherry V2A GFP-RAB-3. NMp3643 was digested with SbfI and MluI and a PstI/BssHII fragment containing the *tetOp cherry V2A GFP-rab-3* from NMp3671 was inserted.

NMp3693 pLF3FShC rab-3p CaTevP. The long *rab-3* promoter amplified from N2 genomic DNA using NMo6408/6409, a synthetic DNA encoding calmodulin (3607, Table S3), a synthetic DNA encoding the TEV protease (3608, Table S3) and the *tbb-2* 3’ UTR amplified from pDD363 using NMo6406/6407 were co-assembled using a SapI GG reaction.

NMp3694 DR274 AAG GTA tbb-2 3’UTR. The *tbb-2* 3’UTR was amplified from NMp3617 with NMo6577/6578, digested with EcoRI and HindIII and inserted into similarly digested NMp3055.

NMp3695 DR274 TGG ATG TetO 7X ∆pes-10. The *tetO* 7X promoter was amplified from NMp3684 using NMo6575/6576, digested with EcoRI and HindIII and inserted into similarly digested NMp3055.

NMp3696 DR274 CT-NT mcs. NMp3591 was digested with BsaI and the ds oligonucleotide NMo6579/6580 was inserted by ligation.

NMp3697 DR274 GGT GCG - BsaI. NMp3456 was digested with BamHI and ApaLI, and the small fragment of BamHI/ApaLI digested NMp3467 was inserted.

NMp3698 DR274 5'arm -CT- BsaI. NMp3467 was digested with BamHI and ApaLI, and the small fragment of BamHI/ApaLI digested NMp3468 was inserted.

NMp3700 DR274 5' arm 11X UAS. NMp3467 was digested with BsaI and an 11X *UAS* enhancer fragment was amplified from NMp1238 with NMo6591/6592 was inserted. Although NMp1238 is a '14X' *UAS* clone, the resulting clone contains only 11X *UAS* elements.

NNp3701 DR274 TGG GGT - BsaI. NMp3467 was digested with BamHI and HindIII and the ds oligonucleotide NMo6585/6586 was inserted by ligation.

NMp3702 DR274 AAG GTA - BsaI. NMp3456 was digested with BamHI and ApaLI, and the small fragment of BamHI/ApaLI digested NMp3467 was inserted.

NMp3703 DR274 5' arm lexO 5X. Two ds oligonucleotide pairs encoding *lexO* binding sites, NMo6595/6597 and NMo6596/6598, were inserted into NMp3467 using a BsaI GG reaction.

NMp3704 DR274 5' arm QUAS 5X. Two ds oligonucleotide pairs encoding *QUAS* binding sites, NMo6599/6601 and NMo6600/6602 were inserted into NMp3467 using a BsaI GG reaction.

NMp3706 DR274 CT ∆pes-10. NMp3468 was digested with BsaI and the *∆pes-10* basal promoter amplified from NMp3671 using NMo6593/6594 was digested with BsaI and inserted.

NMp3708 pLAOF3FShC. The vector backbone of NMp3643 was amplified with NMo6605/6606 to remove the *loxP* site and digested with SalI and PstI. The insert of NMp3643 was then reintroduced by ligation into the modified vector as a SalI/SbfI restriction fragment.

NMp3709 DR274 CT-NT mex-5 landing Mos. NMp3696 was digested with BsiWI and PstI, and the *mex-5* landing from NMp3689 was introduced by ligation as a BsiWI/SbfI fragment.

NMp3710 D274 GGT GCG GAL4SK DB. NMp3697 was digested with SapI and a synthetic DNA fragment (3610, Table S3) encoding a *C. elegans* codon optimized version of the *S. kudriavzevii* GAL4 DNA binding domain was digested with SapI and inserted by ligation.

NMp3711 D274 GGT GCG QF DB. NMp3697 was digested with SapI and the *QF* DNA binding domain from pME-QF was amplified using NMo6603/6604, digested with SapI and inserted by ligation.

NMp3713 pLF3FShC Y8A9A.2p rtetR-QFAD. MNp3643 was digested with SpeI and SbfI and the SpeI/SbfI *Y8A9A.2p rtetR-QFAD::P2A::mKate::T2A::tetR-pie1* fragment from NMp3673 was inserted by ligation.

NMp3718 D274 GGT GCG tetR DB. NMp3697 was digested with SapI and the *tetR* DNA binding domain from a synthetic DNA fragment (3714, Table S3) was digested with SapI and inserted by ligation.

NMp3721 DR274 CT-NT mex-5 RCME landing. The intermediate plasmid NMp3709 was was digested with BsiWI and EcoRV, and a fragment lacking the mos left end was amplified from NMp3689 using NMo6581/6582, digested with BsiWI and EcoRV and inserted by ligation. This plasmid has two different *unc-54* 3’ UTRs and the region between them is occasionally deleted during growth. See NMp3746.

NMp3722 D274 GGT GCG LexA DB. NMp3697 was digested with SapI and the synthetic *lexA* DB domain (3715, Table S3) was digested with SapI and inserted by ligation.

NMp3724 pLF3FSHC tetO 7X ∆pes-10 GFP-C1. The *tetO* 7x *∆pes-10* promoter from NMp3695, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3725 pLF3FShC UAS 11X ∆pes-10 GFP-C1. The *UAS* 11X enhancer from NMp3700, the *∆pes-10* basal promoter from NMp3706, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3726 pLF3FShC QUAS 5X ∆pes-10 GFP-C1. The *QUAS* 5X enhancer from NMp3704, the *∆pes-10* basal promoter from NMp3706, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3727 pLF3FShC lexO 5X ∆pes-10 GFP-C1. The *lexO* 5X enhancer from NMp3703, the *∆pes-10* basal promoter from NMp3706, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NMp3694 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3728 DR274 3' arm tbb-2 3’UTR. NMp3470 was digests with SapI and the *tbb-2* 3’UTR was amplified from N2 using NMo6406/6407, digested with SapI, and inserted by ligation.

NMp3729 pLF3FShC mec-4p LexA-QFAD. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6405, the *LexA* DB domain from NMp3722, the *QF* activation domains as a synthetic DNA (3611, Table S3), and the *tbb-2* 3’UTR amplified from NMp3432 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3730 pLF3FShC mec-4p tetR-QFAD. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6405, the *tetR* DB domain from NMp3718, the *QF* activation domains as a synthetic DNA (3611, Table S3), and the *tbb-2* 3’UTR amplified from NMp3432 were co-assembled into NMp3643 using a SapI GG reaction. DH5α containing this plasmid grows slowly*.*

NMp3731 pLF3FShC mec-4p QF. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6405, the *QF* DNA Binding domain and dimerization domain from NMp3711, the *QF* activation domains as a synthetic DNA (3611, Table S3), and the *tbb-2* 3’UTR amplified from NMp3432 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3732 pLF3FShC mec-4p GFP-C1. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6385, *GFP-C1* from pDD372, and the *tbb-2* 3’UTR from NMp3696 were co-assembled into NMp3643 using a SapI GG reaction.

NMp3733 pLF3FShC mec-4 GAL4SK-VP64. The *mec-4* promoter driving *GAL4SK-VP64* was amplified from NMp3617 using NMo6384/6407 and inserted into NMp3643 using a SapI GG reaction.

NMp3734 pLF3FShC mec-4p GAL4SK-QFAD. The *mec-4* promoter driving *GAL4SK-QF* was amplified from NMp3618 using NMo6384/6407 and inserted into NMp3643 using a SapI GG reaction.

NMp3735 DR274 5' arm-CT mec-4p. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6385 was digested with SapI and inserted into SapI digested NMp3698.

NMp3736 DR274 TGG GGT mec-4p. The *mec-4* promoter amplified from N2 genomic DNA using NMo6384/6405 was digested with SapI and inserted into SapI digested NMp3701.

NMp3738 DR274 GGT GCG rtetR. *rtetR* was amplified in two fragments from NMp3681 using NMo6621/6622 and NMo6623/6624, and co-assembled into NMp3697 using a BsaI GG reaction.

NMp3740 DR274 GCG ACG QFAD. The *QF* activation domains as a synthetic DNA (3611, Table S3), digested with SapI and inserted into SapI digested NMp3696.

NMp3741 pLF3FShC mec-4p rtetR-QFAD. The *mec-4* promoter, from NMp3736, the *rtetR-QFAD* DNA binding domain from NMp3738, the *QF* activation domain from NMp3740, and the *tbb-2* 3’UTR from NMp3728 were co-assembled in a SapI GG reaction.

NMp3743 pBS UAS 11X GFP-C1. The *UAS 11X GFP-C1 ttb-2* 3’ UTR fragment from NMp3725 was isolated as a SpeI/XmaI fragmented inserted into similarly digested pBluescript KS(+).

NMp3744 pBS mec-4p rtetR-QFAD. NMp3741 was digested with XmaI and SpeI and the *mec-4p rtetR-QFAD* cassette was inserted into similarly digested pBluescript KS(+).

NMp3746 DR274 CT-NT LoxP FLP FRT FRT3 landing. This *his-58* 3’ UTR was amplified from N2 genomic DNA using NMo6642/6643, digested with NheI and PstI and used to replace the *unc-54* 3’ UTR of *GFP-his-58* of NMp3721 digested with SbfI and NheI.

NMp3749 DR274 CT-NT loxP FRT FRT3 landing. NMp3746 was digests with NheI and SacII to remove the *loxP mex-5 FLP rpl-28 GFP-his-58* portion of the plasmid and replaced with the NheI/SacII *loxP rpl-28p GFP-his-58* fragment of NMp3649 thus removing the *mex-5 FLP* expression cassette from NMp3746.

NMp3751 DR274 AAG GTA act-4 3’UTR. The *act-4* 3’ UTR was amplified from N2 genomic DNA using NMo6650/6651 and inserted into NMp3702 using a BsaI GG reaction.

NMp3752 pLF3FShC mec-4p rtetR-QFAD tetO 7x GFP-C1. The *mec-4p rtetR-QFAD tbb-2* 3’ UTR cassette was amplified from NMp3744 using NMo6384/6647 and the *tetO 7X ∆pes-10 GFP-C1* cassette was amplified from NMo5997/6648 and the *act-4* 3’ UTR from plasmid 3751 were co-assembled in a SapI GG reaction.

NMp3754 pLF3FShC mec-4p gal4-QF UAS 11x GFP-C1. The *mec-4p GAL4SK-QF* cassette from NMp3618 amplified using NMo6384/6649, the *UAS* 11X *GFP-C1* cassette from NMp3743 amplified using NMo5997/6648, and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG Reaction.

NMp3755 pLF3FShC tetO 7X GFP-C1 act-4 3’UTR. The *tetO* 7X *∆pes-10* promoter from NMp3695, *GFP-C1* from pDD372 and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

NMp3756 pLF3FShC tetO 7X mCherry. The *tetO* 7X *∆pes-10* promoter from NMp3695, *mCherry* from NMp3674 and the *tbb-2* 3’ UTR from NMp3694 were co-assembled in a SapI GG reaction.

NMp3767 DR274 GCG ATG mec-7bp. The *mec-7* basal promoter (-154 to +3) (Duggan et al., 1998) was amplified from N2 genomic DNA using NMo6669/6670 and inserted into NMp3468 using a BsaI GG reaction.

NMp3768 DR274 GCG ATG myo-2bp. The *myo-2* basal promoter (-175 to +3) (Okkema et al., 1993) was amplified from N2 genomic DNA using NMo6673/6674 and inserted into NMp3468 using a BsaI GG reaction.

NMp3769 DR274 GCG ATG hsp-16.1bp. The *hsp-16.1* basal promoter (-87 to +3) (Hong et al., 2004) was amplified from N2 genomic DNA using NMo6671/6672 and inserted into NMp3468 using a BsaI GG reaction.

NMp3770 DR274 TGG GCG tetO 7X.The *tetO* 7X enhancer was amplified from NMp3724 with NMo6575/6668, and digested with EcoRI and HindIII and inserted into similarly digested NMp3055.

NMp3773 DR274 CT-NT -BsaI II. NMp3055 was digested with EcoRI and HindIII and the ds oligonucleotide NMo6718/6719 was inserted by ligation. This plasmid is similar to NMp3591, but corrects a limitation of that vector (see NMp3591).

NMp3774 pLFF3SHC tetO 7X ∆mec-7p GFP-C1. The *tetO* 7X enhancer from NMp3770, the *mec-7* basal promoter from NMp3767, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NM3694 were co-assembled in a SapI GG reaction.

NMp3775 pLFF3SHC tetO 7X ∆myo-2p GFP-C1. The *tetO* 7X enhancer from NMp3770, the *myo-2* basal promoter from NMp3768, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NM3694 were co-assembled in a SapI GG reaction.

NMp3776 pLFF3SHC tetO 7X ∆hsp-16p GFP-C1. The *tetO* 7X enhancer from NMp3770, the *hsp-16.1* basal promoter from NMp3769, *GFP-C1* from pDD372, and the *tbb-2* 3’ UTR from NM3694 were co-assembled in a SapI GG reaction.

NMp3777 DR274 3'arm tbb-2 UTR. The *tbb-2* 3’ UTR was amplified from N2 genomic DNA with NMo6676/6407, digested with SapI, and inserted into SapI digested NMp3470.

NMp3791 DR274 GGT GCG QF2n DB. The native DNA binding domain found in *QF2* (Riabinina et al., 2015) was amplified from NMp3731 using NMo6712/6713, and inserted into NMp3697 using a BsaI GG reaction.

NMp3800 DR274 CT-NT QF2 AD. A codon optimized version of the activation domain found in *QF2* was amplified from the synthetic *QF* activation domain DNA fragment (3611, Table S3) using NMo6710/6711, digested with SapI and inserted into SapI digested NMp3686.

NMp3808 DR274 CT-NT linker-QFAD. A flex linker domain was added to the *QF* activation domain from NMp3800 by amplifying using NMo6723/6724 and inserting the fragment into NMp3773 using a BsaI GG reaction.

NMp3810 pLF3FShC mec-4p QF act-4 3’UTR. The *mec-4* promoter from NMp3735, *QF* amplified from *wySi377* genomic DNA in two fragments using NMo6728/3729 and NMo6730/6731, and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

NMp3811 pLF3FShC mec-4p QF2 act-4 3’UTR. The *mec-4* promoter from NMp3735, *QF2* DNA binding domain amplified from NM3791 using NMo6728/6133, the flex linker and *QF* activation domain and from NM3808 amplified using NMo5008/6734, and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

NMp3813 pLF3FShC mec-4p LexA-L-QF act-4 3’UTR. The *mec-4* promoter from NMp3735, *lexA* DNA binding domain amplified from NM3722 using NMo6733/6133, the flex linker and *QF* activation domain and from NM3808 amplified using NMo5008/6734, and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

NMp3814 pLF3FShC mec-4p rtetR-L-QF act-4 3’UTR. The *mec-4* promoter from NMp3735, *rtetR* DNA binding domain amplified from NM3738 using NMo6732/6133, the flex linker and *QF* activation domain and from NM3808 amplified using NMo5008/6734, and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

NMp3817 DR274 GGT GCG tetR(iS) DB. Two stop codons were introduced into the artificial intron in the *tetR* coding sequences of NMp3718 by amplifying the plasmid using NMo6736/6737, kinasing the product, and re-ligating. The stop codons were introduced because I was unable to create clones in *E. coli* DH5α expressing *tetR*-*linker-QF* containing the synthetic *tetR* gene into ampicillin resistant plasmids.

NMp3821 DR274 FP tetR(iS)-L-QF. The *tetR-linker-QF* clone was assembled in NMp3469 from 3 fragments in a BsaI GG reaction. The N-terminal part of the DNA binding domain was amplified using NMo6745/6746 from the synthetic *tetR* gene in NMp3718, the middle region of the *tetR* DNA binding domain was amplified from the *rtetR* sequences of NMp3814 with oligos NMo6747/6748 containing mis-matches to switch the coding from *rtetR* to *tetR*, and the C-terminal region of the *tetR* DNA binding domain, the linker region and the *QF* activation domain were amplified from NMp3814 using NMo6749/6750.

NMp3823 pLF3F mec-4p tetR-L-QF act-4 3’ UTR. The *mec-4* promoter from NMp3735, *tetR-L-QF* from NMp3821 and the *act-4* 3’ UTR from NMp3751 were co-assembled in a SapI GG reaction.

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