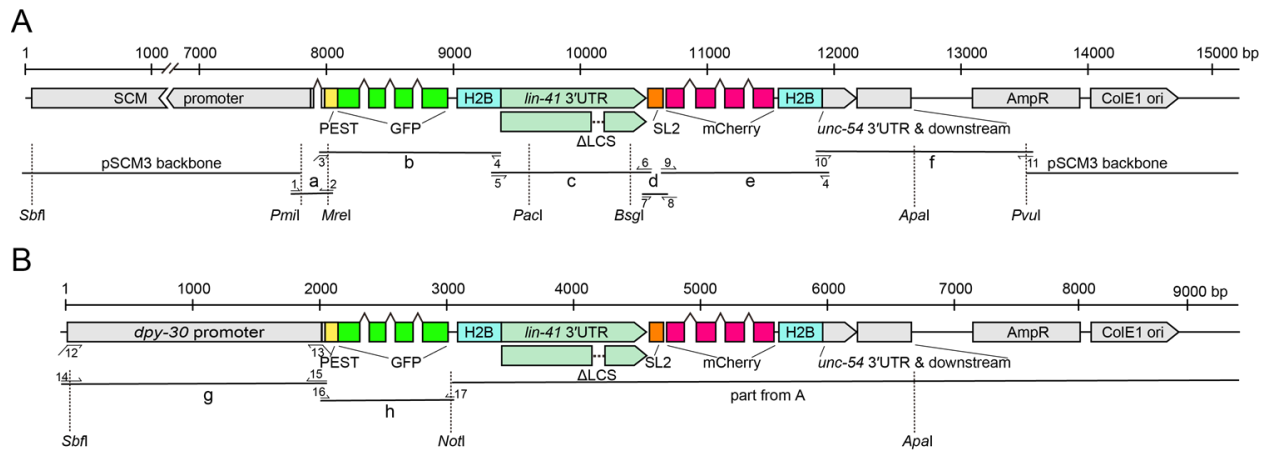


Supplementary Figure S2



Supplementary Figure S2. Design of dual color operon reporters.

(A) For plasmids SCMp::GFP(PEST)::H2B::lin-41_3'UTR::SL2::mCherry::H2B::unc-54_3'UTR and SCMp::GFP(PEST)::H2B::lin-41_3'UTR_ΔLCS::SL2::mCherry::H2B::unc-54_3'UTR, fragment 'a' containing the 3' end of the SCM promoter region with a *PmlI* site, an artificial intron (IVS.A, Fire lab *C. elegans* vector kit), and part of the GFP::PEST sequence with a *MreI* site, was amplified by PCR from pSCM3::GFP. Fragments 'b' and 'c' were amplified from HW769, 'd' from N2 genomic DNA, and 'e' from pCM1.151 to form the core fluorescence operon reporter. Fragment 'f' was amplified by PCR from pSCM3::GFP (a gift from Dr. Hitoshi Sawa) and contains the *unc-54* 3'UTR and a *PvuI* site in the AmpR gene of the vector. Then these fragments were linked by multiple Gibson assembly and ligation reactions, and finally cloned into the pSCM3 backbone at the *PmlI/PvuI* sites. To modify the *lin-41* 3'UTR, the wild-type *PacI/BspI* section was exchanged with that from a previously cloned construct carrying *lin-41* 3'UTRΔLCS (LCS, *let-7* complementary site) from HW783.

(B) For plasmids dpy-30p::GFP(PEST)::H2B::lin-41_3'UTR::SL2::mCherry::H2B::unc-54_3'UTR and dpy-30p::GFP(PEST)::H2B::lin-41_3'UTR_ΔLCS::SL2::mCherry::H2B::unc-54_3'UTR, a 2.0 kb *dpy-30* promoter was amplified by PCR using Gateway compatible primers 12 and 13 from N2 genomic DNA and then cloned into pDONR P4-P1r by a BP reaction. To be adapted as an operon reporter, it was re-amplified by PCR using primers 14 and 15 to produce fragment 'g'. Fragment 'h' containing an overlapping sequence with fragment 'g' and the *NotI* site after GFP and prior to H2B was amplified by PCR using the construct in (A) as the template. Then fragments 'g' and 'h' were ligated by Gibson assembly and used to replace the SCM promoter and IVS.A artificial intron in both plasmids in (A) by *SbfI/NotI* restriction enzyme cloning. These operon reporters were then cloned into the pCG150_double_phiC31_attB destination vector at the *SbfI/ApaI* sites for phiC31-mediated single copy insertion. Primers used to construct these reporters are shown as half arrows and are listed in **Supplementary Table S3**.