

Resource Summary Report

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KG#230

RRID:Addgene_110925

Type: Plasmid

Proper Citation

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Plasmid Information

URL: <http://www.addgene.org/110925>

Proper Citation: RRID:Addgene_110925

Insert Name: unc-129 promoter

Organism: Caenorhabditis elegans

Bacterial Resistance: Ampicillin

Defining Citation: [PMID:19797080](#)

Vector Backbone Description: Backbone Size:2632; Vector Backbone:pUC; Vector Types:Bacterial Expression; Bacterial Resistance:Ampicillin

Comments: Unique multi-cloning sites: Nhe I/ Sal I/ Kpn I/ Age I/ Bgl II Used Pfu Ultra polymerase and primers engineered with restriction sites to amplify the 2.6 Kb unc-129 promoter from N2 genomic DNA and cloned it into Pst I/ Bam HI cut pPD96.52 (C. elegans body wall muscle expression vector; this digestion will remove the myo-3 promoter in 2 fragments and leave the 3.7 kb vector). Transformed into XL1-Blue electrocompetent cells. Miniprepmed 4 clones to find those with correct size insert and vector, then submitted 1 clone for sequence. Chose one clone that has correct sequence and made glycerol stock. Features of the expression construct: This expression construct has a promoter (unc-129::) that will drive strong and specific expression (of whatever gene is inserted in the MCS) in 9 of the DA/ DB cholinergic neurons of the ventral nerve cord. unc-129 promoter sequence includes the region from the native Sma I site (2.6 Kb upstream of the start codon) down to the 7th nucleotide before the A of the ATG. This includes a 19 bp 5' UTR (out of 25 bp 5' UTR total). This promoter is a shortened version of the unc-129 promoter. This shortened version drives expression in 9 ventral cord motor neurons that Sieburth et al identified as the DA's. The promoter itself is described in Colavita et al., 1998, although they claim expression in both DA's and DB's. The DA motor neurons form NMJ's in the dorsal nerve cord and receive synaptic inputs in the ventral cord (therefore proteins localized to the ventral cord would be dendritically localized/ postsynaptic). Other

features of the expression vector include a "decoy" 5' to the promoter to reduce non-specific expression in the gut (see Fire Lab 1995 Kit documentation for description), the unc-54 3' end including the poly A addition signal, and 3 introns (1 in 5' UTR, 1 just upstream of the unc-54 3' end, and 1 inserted in the unc-54 3' end). These introns help provide more uniform and robust expression, especially if you are expressing a cDNA with no introns as opposed to a gene with introns. Note: this vector can be converted to a vector that fuses GFP, YFP, or CFP onto the C-terminus of the protein as follows: Remove the ~1000 bp (Kpn I or Age I) + (Spe I or BsiW I [expensive, 55 C cutter with 50% activity at 37 C; heat inactivate 80 C] or Apa I) fragment containing the unc-54 3' control region from this plasmid and replace it with the ~1800 bp like-digested fragment containing 3-intron S65C GFP (see pPD94.81 in Fire Lab plasmids), YFP (see pPD136.64 in Fire Lab plasmids) or CFP (see pPD136.61 in Fire Lab plasmids) + the unc-54 3' UTR and control region. Note if Spe I is used to cut these Fire Lab vectors, you get 3 bands: 1100, 1800, and 4000 (total size 6.9 Kb). The 1800 bp band is the XFP-containing band. For C-terminal fusions, remember to leave the stop codon off of the upstream protein. For the above-mentioned Fire Lab vectors, the reading frame for the downstream XFP relative to the Kpn I and Age I sites is as follows (triplets are the reading frame codons): Kpn I G GTA CCG GT Age I Alternatively, use Gibson Assembly/ NEBuilder to insert any genetically encoded fluorescent protein at the N- or C-terminus of your protein.

Plasmid Name: KG#230

Record Creation Time: 20220422T221539+0000

Record Last Update: 20260815T080144+0000

Ratings and Alerts

No rating or validation information has been found for KG#230.

No alerts have been found for KG#230.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.