

Resource Summary Report

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KG#371

RRID:Addgene_110882

Type: Plasmid

Proper Citation

RRID:Addgene_110882

Plasmid Information

URL: <http://www.addgene.org/110882>

Proper Citation: RRID:Addgene_110882

Insert Name: unc-129 promoter

Organism: Caenorhabditis elegans

Bacterial Resistance: Ampicillin

Defining Citation: [PMID:23633144](#)

Vector Backbone Description: Backbone Size:2610; Vector Backbone:pUC; Vector Types:Bacterial Expression; Bacterial Resistance:Ampicillin

Comments: We used Herculase II polymerase and primers engineered with restriction sites to amplify the 1.2 Kb ctns-1 cDNA "a" isoform (minus its stop codon and in frame with downstream RFP) from KG#354 and cloned it into Nhe I/ Age I cut KG#240 (7.2 Kb; unc-129::____-mCherry). Transformed into XL1-Blue electrocompetent cells. Qiagen miniprepmed 2 clones to find those with correct insert, then confirmed insert by sequencing. Reference: Edwards SL, Yu S, Hoover, CM, Phillips BC, Richmond JE, and Miller KG. (2013). An Organelle Gatekeeper Function for Caenorhabditis elegans UNC-16 (JIP3) at the Axon Initial Segment. Genetics. 194(1): 143-161.PMCID: PMC3632462. Features of the construct: ctns-1a is a marker for lysosomes. An AAAA precedes the start codon for a good consensus ribosome binding site. 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 DA/ DB cholinergic neurons of the ventral nerve cord. The 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). The stop codon is left off of the ctns-1 cDNA for in-frame fusion to the downstream RFP (mCherry). The Age I site encodes 2 amino acids that are in frame with the downstream RFP. There is an CAG following the restriction site that is not part of the following intron. The restriction site + these 3 nucleotides will code

for the amino acids Thr, Gly, and Gln and those last 3 nucleotides (CAG) provide a good consensus for preceding an intron. After this is the 44 bp intron 3 from T07H6.4 followed by the mCherry cDNA sequence (711 bp; no further introns; this is the mCherry cDNA obtained directly from the Tsien lab at UCSD) and stop codon, then the unc-54 3' control region with the 2 introns (1 just upstream of the unc-54 sequence and 1 in the unc-54 sequence) in it because the Apa I site is the 3' boundary of all of this. This vector also includes the 5' UTR intron found in most Fire lab vectors. Another feature 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).

Plasmid Name: KG#371

Record Creation Time: 20220422T221539+0000

Record Last Update: 20260919T090516+0000

Ratings and Alerts

No rating or validation information has been found for KG#371.

No alerts have been found for KG#371.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.