Resource Summary Report

Generated by RRID on Aug 17, 2026

pcDNA3.1(+)mGAT1-577-YFP-578CT

RRID:Addgene_41681
Type: Plasmid

Proper Citation

RRID:Addgene_41681

Plasmid Information

URL: http://www.addgene.org/41681

Proper Citation: RRID:Addgene_41681

Insert Name: Mus musculus GABA transporter 1

Organism: Mus musculus

Bacterial Resistance: Ampicillin

Defining Citation: PMID:19948998

Vector Backbone Description: Backbone Marker:Invitrogen; Backbone Size:5428; Vector Backbone:pcDNA3.1(+); Vector Types:Mammalian Expression; Bacterial Resistance:Ampicillin

Comments: To generate the fluorescent mutants mGAT10XFP and mGAT1XFP* through mGAT1XFP45, the wild-type mGAT1 open reading frame (ORF) was subcloned without its original stop codon into the HindIII and EcoRI sites of the pcDNA3.1(+) expression vector multiple cloning site (MCS). XFP ORFs were then subcloned downstream from and in frame with the mGAT1 ORF at the NotI and XbaI sites of the pcDNA3.1(+) MCS. This resulted in a 12–amino acid spacer between the end of the mGAT1 sequence and the beginning of the fluorophore. The depositors modified a method for the integration of PCR fragments without the use of restriction enzymes (Geiser et al., 2001) to add the final 3, 8, 20, 28, or 45 codons of the human GAT1 (hGAT1) ORF. These were amplified from a source plasmid using the proof-reading PfuTurbo Cx Hotstart polymerase with 5? and 3? extensions corresponding to the 20–22-nt regions that flanked the intended site of insertion, such that the PCR product integrated in-frame immediately after the fluorophore sequence when used as the primers in a subsequent QuikChange II XL mutagenesis PCR reaction. For mGAT1XFP*, the depositors simply added a GTC codon for Val after the fluorophore ORF. The attached image displays the protein sequences of the modified regions of mGAT1 for each fluorescent construct. mGAT10CFP and mGAT10YFP repeated the fusion design of mGAT10GFP but with the fluorophore exchanged as

annotated. The three C-terminal residues of the mGAT0XFP fusions are -YKI-CO2?, which comprises a broadly defined consensus PDZ class II–interacting motif (X-?-X-?, where ? designates a hydrophobic residue and X any residue) (Sheng and Sala, 2001; Hung and Sheng, 2002). The depositors searched the Ensembl databases using Biomart (http://www.ebi.ac.uk/biomart) (Spudich et al., 2007) and applied the GO:0005886 “plasma membrane” cellular component filter. The search identified no known membrane proteins possessing the -YKI-CO2? C-terminal sequence. In the mGAT1XFP* constructs, the depositors defined the terminal residue P(0) more narrowly, changing the terminal isoleucine residue present in mGAT10XFP to a valine in mGAT1XFP*. The resulting C-terminal sequence, -YKV-CO2?, reconstituted a functional PDZ class II–interacting motif present in Ephrin B receptors, a class that relies on interactions with the PDZ domain–containing proteins for clustering (Torres et al., 1998; Brückner et al., 1999; Lin et al., 1999; Madsen et al., 2005). Other constructs in the C-terminal XFP fusion series, mGAT1XFP3, mGAT1XFP8, mGAT1XFP20, mGAT1XFP28, and mGAT1XFP45, had the most C-terminal 3, 8, 20, 28, or 45 residues of the hGAT1 appended after the mGAT1XFP fusion. The differences in nucleotide sequence between the hGAT1 and mGAT1 C termini were a useful source of positive identification when the depositors analyzed the clones during construction. PCR integration was applied to amplify and insert EYFP or ECFP directly between residues R565 and L566, I570 and Q571, or V577 and R578 of mGAT1 to generate the mGAT15xxXFP5xxCT constructs. The site of XFP insertion in GAT1 is highlighted in the nomenclatures for these constructs by residue numbers flanking the fluorophore, and the “CT” denotes that the insertion occurs within the C terminus. Please see the associated article for more detailed information regarding construct creation and usage.

Plasmid Name: pcDNA3.1(+)mGAT1-577-YFP-578CT

Relevant Mutation: “Monomerizing” YFP A206K mutation; YFP inserted directly between residues V577 and R578 of mGAT1

Record Creation Time: 20220422T222216+0000

Record Last Update: 20260815T081447+0000

Ratings and Alerts

No rating or validation information has been found for pcDNA3.1(+)mGAT1-577-YFP-578CT.

No alerts have been found for pcDNA3.1(+)mGAT1-577-YFP-578CT.

Data and Source Information

Source: Addgene

Usage and Citation Metrics

We found 1 mentions in open access literature.

Listed below are recent publications. The full list is available at RRID .

Tai Y, et al. (2019) Enhanced mitochondrial pyruvate transport elicits a robust ROS production to sensitize the antitumor efficacy of interferon-? in colon cancer. Redox biology, 20, 451.