

Resource Summary Report

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pTRE-T2-miR-PURO

RRID:Addgene_102646

Type: Plasmid

Proper Citation

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Plasmid Information

URL: <http://www.addgene.org/102646>

Proper Citation: RRID:Addgene_102646

Bacterial Resistance: Ampicillin

Vector Backbone Description: Backbone Marker:Markus Ralser (Addgene plasmid # 19407), Clontech = pTRE-T2; Vector Backbone:pTRE-Tight2; Vector Types:Mammalian Expression, RNAi; Bacterial Resistance:Ampicillin

Comments: In fusion cloning was used to insert a synthetic DNA template containing the miR cassette from the pCDNA 6.2-GW/miR into the EcoRI and XbaI site of pTRE-T2-Tight. I engineered two BsaI sites into the miR cassette so users can digest the vector with BsaI for directional cloning of Blockit microRNAs using the Block-IT RNAi designer engine from Invitrogen. To make the BsaI sites unique I mutated out BsaI sites in the original pTRE-T2 Tight vector and also removed XhoI and XbaI sites by site-directed mutagenesis from the pTRE-T2 vector and the corresponding puromycin/hygromycin resistance genes. I also dropped in a unique NheI and EagI restriction site between the ampicillin gene and the pMB101 origin of replication sequence so that the puromycin and hygromycin genes could be placed into the pTRE-T2-miR vector with the infusion cloning method. Investigators can remove the miR cassette from the pTRE-T2 puromycin/hygromycin vector and using cloning methods to insert any cDNA of interest into the site for doxycycline-inducible expression of the target cDNA. A single vector can be utilized from the production of tetracycline inducible miRNAs or cDNAs. For puromycin use .25 micrograms/ml to .50 micrograms/ml to get clones after a couple of weeks. Growth response curve tested in 293HEK cells. Suggest methylation deficient cells from NEB to alleviate methylation sensitive restriction sites like XbaI. However not recommended for long-term propagation and storage.

Plasmid Name: pTRE-T2-miR-PURO

Record Creation Time: 20220422T221457+0000

Record Last Update: 20260902T040950+0000

Ratings and Alerts

No rating or validation information has been found for pTRE-T2-miR-PURO.

No alerts have been found for pTRE-T2-miR-PURO.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.