

Resource Summary Report

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pET MBP TEV expression vector with BrioBrick polypromoter restriction sites (14-Q)

RRID:Addgene_48311

Type: Plasmid

Proper Citation

RRID:Addgene_48311

Plasmid Information

URL: <http://www.addgene.org/48311>

Proper Citation: RRID:Addgene_48311

Bacterial Resistance: Ampicillin

Vector Backbone Description: Backbone Size:4151; Vector Backbone:pET; Vector Types:Bacterial Expression; Bacterial Resistance:Ampicillin

Comments: This plasmid is an empty vector to be used with a LIC cloning protocol. It has a TEV cleavable MBP tag on the N-terminus and Amp resistance. To clone into this vector, add LIC fusion tags to the 5' end of your PCR primers. Forward - 5'TACTTCCAATCCAATGCA3' Reverse - 5'TTATCCACTTCCAATGTTATTA3' Linearize the plasmid with SspI and gel purify. When digesting the DNA with T4 polymerase for LIC, use dCTP for insert and dGTP for vector. The Series-14 plasmids were designed to overcome the unpredictable expression patterns of genes from a polycistronic message. As mentioned in the Series-2 and Series-9 polycistronic sections, often genes that express well from a single gene mRNA do not express at the same level when placed in a polycistronic message next to other genes. This effect is likely due to mRNA structure interfering with access to the ribosome binding site. When we first made our polypromoter plasmids we found that we could clone multiple genes together in the Xl1-Blue cells so that each possess a T7 promoter, followed by a Lac operator, followed by a ribosome binding site, followed by your open reading frame (T7-LacO-RBS-yORF). However, these multi-gene polypromoter plasmids were unable to be transformed into an expression strain such as BL-21 or Rosetta2. We then figured out that we needed a RecAexpression strain (like Rosetta-Blues) to successfully propagate our polypromoter plasmids. Although Rosetta-Blue cells propagated our plasmids, expression was not so great and we didn't want to rely on a specialized strain to perform our expression. Finally, we figured out that it was the LacO that follows the T7 promoter that was causing plasmid instability in our polypromoter system. Removal of the LacO allowed us to express our polypromoter plasmids in Rosetta2 cells and we have had much success with these plasmids since.

Much like our Series- 2 plasmids, the Series-14 plasmids are going to give a substantial amount of leaky expression. However, even with this shortcoming, we have found Series-14 to be a valuable resource for expressing multi-protein complexes. Cloning into the Series-14 plasmids and subsequent biobrick subcloning is performed exactly as described for the Series-9 plasmids. More information on this vector can be found through <http://qb3.berkeley.edu/qb3/macrolab/>

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Record Creation Time: 20220422T222248+0000

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Ratings and Alerts

No rating or validation information has been found for pET MBP TEV expression vector with BrioBrick polypromoter restriction sites (14-Q).

No alerts have been found for pET MBP TEV expression vector with BrioBrick polypromoter restriction sites (14-Q).

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](#) .

Lyles KV, et al. (2022) HupZ, a Unique Heme-Binding Protein, Enhances Group A Streptococcus Fitness During Mucosal Colonization. *Frontiers in cellular and infection microbiology*, 12, 867963.