BBF RFC #111: Inducible expression vector with His tag fusion for purification of RFC25 parts

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1. Purpose

This Request For Comments (RFC) describes a new expression vector based on the pET expression system and designed for BioBrick RFC25 parts. This design includes all the features of pET expression vectors with a new origin of replication, resistance marker and Multiple Cloning Site (MCS). This MCS contains new restriction sites and a 5' poly-histidine tag that allows the purification of expressed RFC25 parts. Previous attempts have been prevented by restriction site incompatibility, but using RFC25 parts and their restriction sites enables 5' fusion to these parts. This new expression vector enables the cloning of RFC25 parts with a 5' His tag in a single step. This allows the inducible expression of these parts and the easy purification of protein products.

2. Relation to other BBF RFCs

BBF RFC111 does not update or replace any earlier RFC. As RFC111 describes a vector for the use of RFC25 parts, it is fully compatible with these parts and thus extends RFC25.

3. Copyright and License Notice

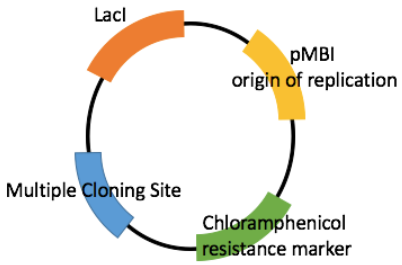
4. Motivation

This RFC was designed to solve the problem of purifying protein products from BioBrick compatible parts. No other expression vector previously reported provides users with the advantage of inducibility, integrated His tag fusion, and the possibility to clone with BioBrick restriction enzymes. As one of the most common BioBrick standard, RFC25 compatibility was chosen because RFC10 prefix and suffix introduce stop codons that disable the His tag fusion. This expression vector, designed by the 2015 iGEM team from Concordia University, is inspired by the pET series of expression vectors and follows

BioBrick compatibility rules. Hence it is named pET15BB. This part has also been submitted to the iGEM registry under the accession number Bba_K1830000.

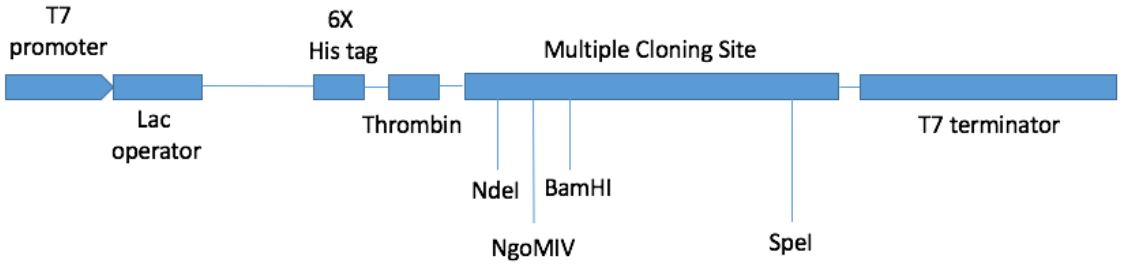
5. Description

The design of pET15BB is based on the high-copy number backbone pSB1C3. This backbone provides the construct with a selection marker for Chloramphenicol resistance, as well as the *Escherichia coli* origin of replication pMBI. In addition to lacking RFC25 restriction sites, the high-copy number of the backbone is advantageous for overexpression of cloned genes.



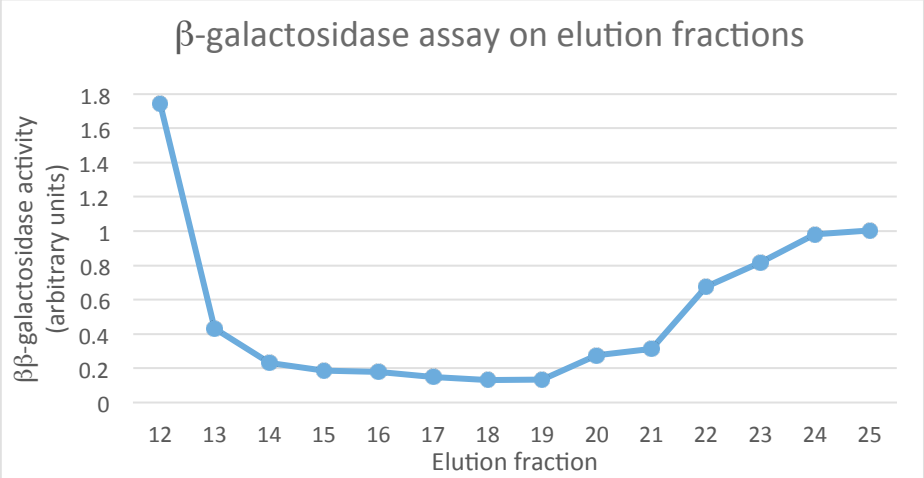
The inducibility of pET15BB is enabled by the addition of the Lac inducer gene (LacI) into the construct. Along with the lac operator located in the expression cassette, protein expression is under the control of the synthetic inducer IPTG.

The design of the expression cassette is also based on the pET15b expression vector. It contains a T7 promoter with the lac operator, a 6X his tag, a thrombin tag, a Multiple Cloning Site (MCS) and a T7 terminator. The MCS has been designed specifically to receive any RFC25 part. A 5' NgoMIV and a 3' SpeI were chosen because NgoMIV does not introduce a stop codon which would disrupt the His tag fusion. Also, SpeI and NgoMIV do not produce complementary overhangs and make directional cloning possible. Other restriction sites found in pET vectors have been kept in the MCS for increased flexibility in cloning design. In addition to this, the entire expression cassette is flanked by EcoRI and PstI restriction sites. Therefore, a different expression cassette can easily be designed and inserted.



6. Results

pET15BB was tested by cloning the LacZ gene into the MCS with RFC25 prefix and suffix. The plasmid was transformed into *E. coli* BL21(DE3) strain. Cultures were grown to OD 0.5 and expression was induced with 1mM IPTG for 6 hours. The cultures were lysed by sonication and lysates were purified by nickel affinity purification. 1mL elution fractions were collected and were assayed for β-galactosidase activity with o-nitrophenyl-β-galactoside (ONPG). Activity was monitored by the increase in absorbance at 420nm for 2 minutes.



Endogenous β-galactosidase activity was detected in early elution fractions 1 to 13 and decreased to almost null levels. Addition of 250mM imidazole to the elution buffer after fraction 20 leads to an increase in β-galactosidase activity in all later fractions. This is due to His-tagged enzymes that elute from the column at high imidazole concentration only. This results confirms the functionality of pET15BB to induce expression and purify proteins.

7. Advantages

This newly designed expression vector solves the problem of purifying proteins produced from BioBrick parts with a minimal number of cloning steps. The His tag fusion integrated in the vector prevents the user from having to add a tag beforehand. Moreover, having the BioBrick cut sites required for insertion reduces the additional step of modifying the restriction sites flanking the insert. This is especially useful to iGEM teams who need to purify proteins as they can submit their parts and clone them for expression with the same prefix and suffix design.

The high-copy number characteristic of this expression vector is also a useful feature for protein purification. Indeed, it increases the number of gene copies available for translation, which increases protein yield.

Finally, the design of pET15BB provides great flexibility in the cloning design. In addition to RFC25-specific restriction sites that facilitates BioBrick cloning, other widespread restriction sites are found in the MCS. Moreover, the two restriction sites flanking the entire expression cassette allow the integration of an alternative expression cassette. This opens the way to an expression vector toolbox with standardized parts, enabling its complete customization. For instance, one could easily integrate a given promoter or tag, and even choose a different gene expression system.

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References

Dubendorf, J. W., & Studier, F. W. (1991). Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor. *Journal of molecular biology*, *219*(1), 45-59.