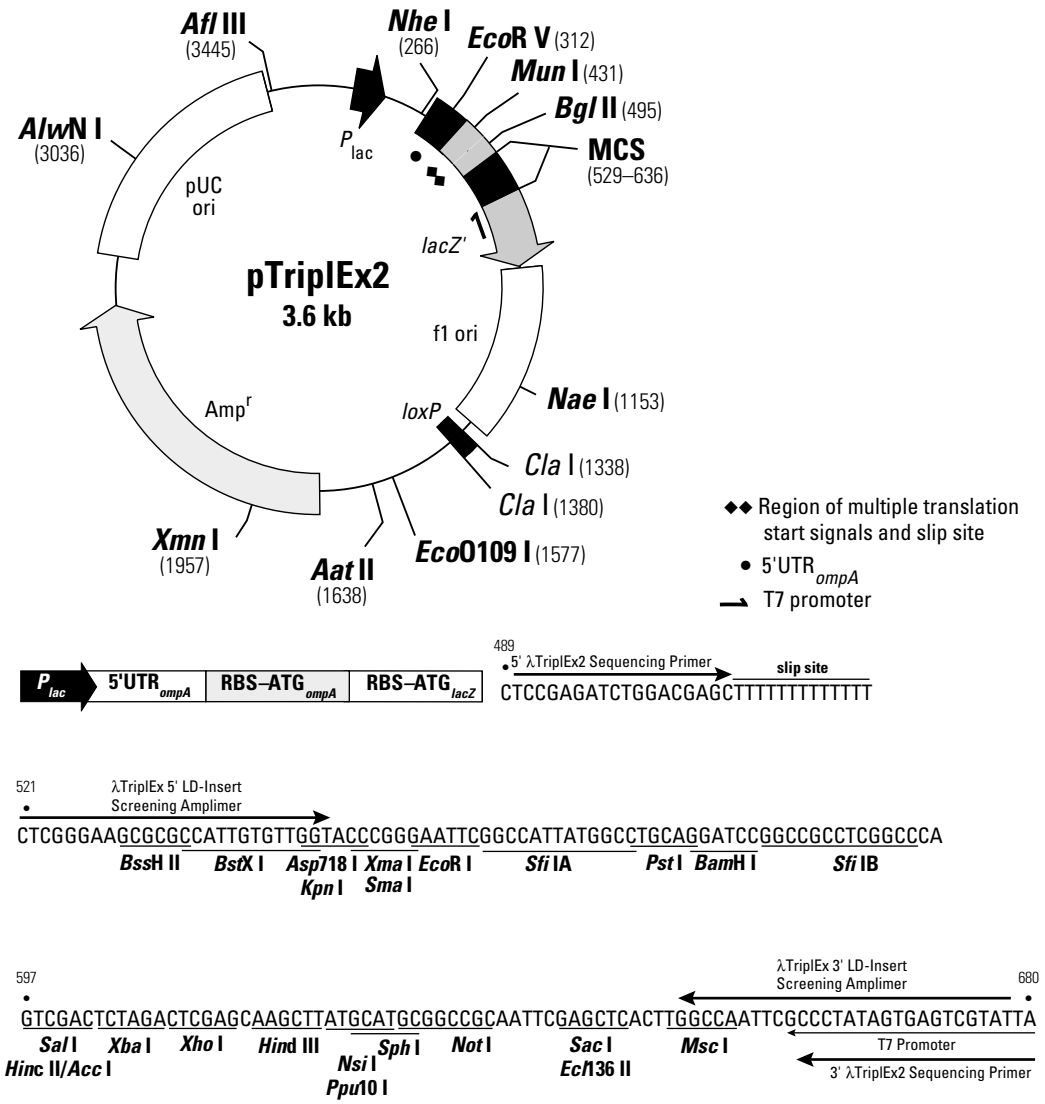

Restriction map and multiple cloning site (MCS) of pTriplEx2. Unique restriction sites are bold.

Description

pTriplEx2 has the *E. coli lac* promoter and operator to provide regulated expression of inserts in *E. coli* hosts expressing the lac repressor (*lacI*^{q}). The 5' untranslated region (UTR) from the *E. coli ompA* gene stabilizes the mRNA, thereby increasing expression. pTriplEx2 incorporates a triple-reading-frame translation cassette consisting of translation initiation signals from the *E. coli ompA* and *lacZ* genes, in two different reading frames, followed by a transcription/translation slip site. Downstream of this cassette is the pTriplEx2 MCS which is embedded within the *lacZ* α-peptide allowing clones with inserts to be identified by blue/white screening in an appropriate host strain. The T7 RNA polymerase promoter downstream of the MCS allows production of single-stranded RNA *in vitro* for use as a probe. In the presence of helper phage, the f1 origin in pTriplEx packages the noncoding strand of the *lacZ* gene into phage particles, and this single-stranded DNA can be used for sequencing or mutagenesis procedures. The ampicillin resistance gene and pUC origin of replication allow selection and propagation, respectively, of pTriplEx2 in *E. coli*.

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Triple-reading-frame translation preceding the *Eco*R I site in pTriplEx2. The fusion protein expressed from inserts cloned into the *Eco*R I site of λTriplEx2 or pTriplEx2 will contain either 34–35 or 53–54 amino acids encoded by the vector, depending on which start codon and which reading frame is used. In general, each insert is expressed in all three reading frames in each cell.

Location of features

- *lac* promoter: 94–177
  - CAP-binding site: 110–123
  - –35 region: 142–147; –10 region: 166–171
  - Transcription start point: 178
  - *lac* operator: 178–198
- 5'-untranslated region (UTR) of *E. coli ompA* gene: 276–413
- α-peptide of *E. coli* β-galactosidase: 469–849
- Triple-reading-frame translation cassette
  - Translation initiation signals from *E. coli ompA* gene:
    - Ribosome binding site: 398–405; Start codon (ATG): 414-416
  - Translation initiation signals from *E. coli lacZ* gene:
    - Ribosome binding site: 458–461; Start codon (ATG): 469–471
  - Transcription/translation slip site: 508–520
- MCS: 529–665
- Phage promoters
  - SP6 RNA polymerase promoter: 238–257; Transcription start point: 255
  - T7 RNA polymerase promoter: 680–661; Transcription start point: 663
- f1 DNA origin: 1299–844
  - [The non-coding strand of the *lacZ* gene is packaged into phage particles.]
- *loxP* recombination site: 1359–1392
- Ampicillin-resistance gene:
  - Promoter: –35 region: 1718–1723; –10 region: 1741–1746
  - Transcription start point: 1753
  - Ribosome binding site: 1776–1780
  - β-lactamase coding sequences: start codon: 1788–1790; stop codon: 2646–2648
  - β-lactamase signal peptide: 1788–1856
  - β-lactamase mature protein: 1857–2645
- pUC plasmid replication origin: 2796–3439

Primer locations

- λTriplEx 5' LD-Insert Screening Amplimer (#9107-1): 521–546
- λTriplEx 3' LD-Insert Screening Amplimer (#9107-1): 678–651
- λTriplEx 5' sequencing primer (5' λTriplEx2 Sequence): 490–507
- λTriplEx 3' sequencing primer (3' λTriplEx2 Sequence): 680–661

Propagation in *E. coli*

- Suitable host strains: JM109, XL1-Blue, and other strains carrying *lacI*^{q}.
- Selectable marker: plasmid confers resistance to ampicillin (50–100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1