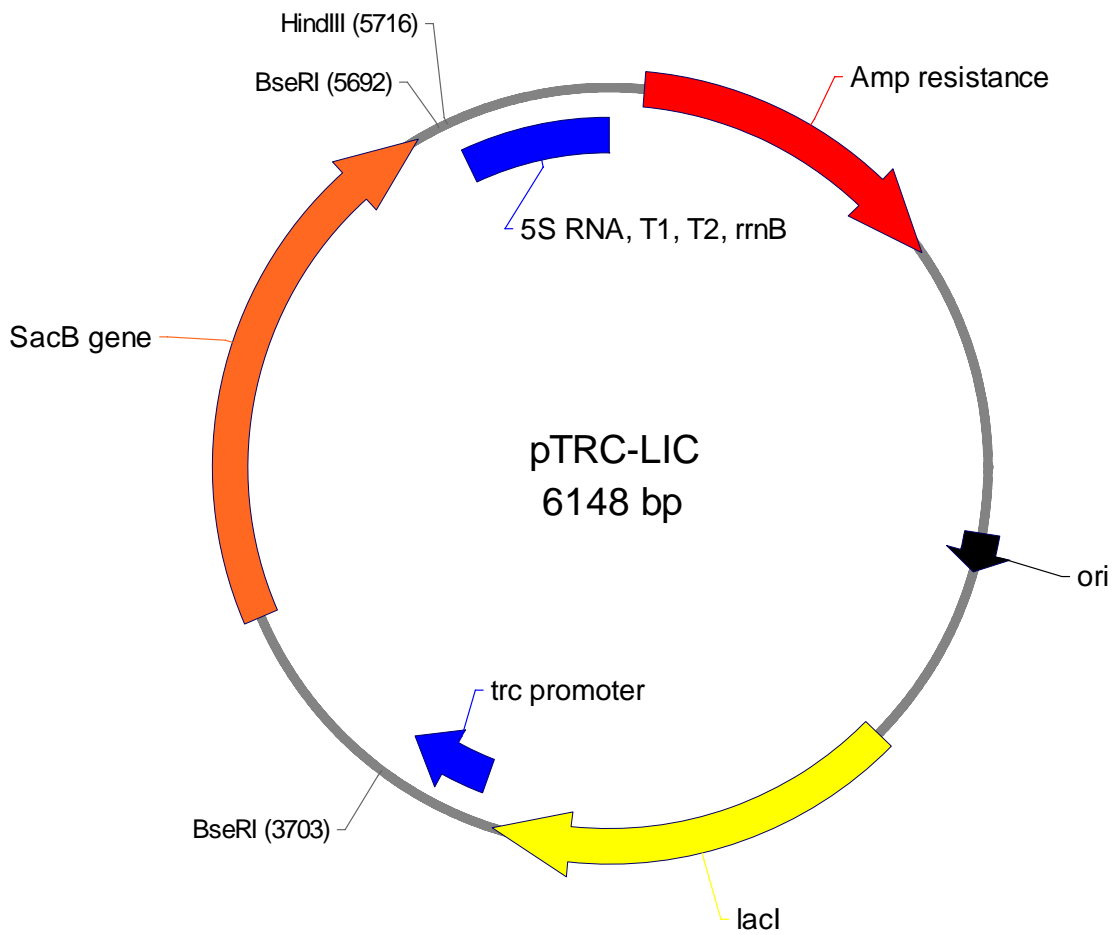


## pTRC-LIC Vector (GenBank accession EF460847)

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto
Description	The pTRC-LIC vector was derived from expression plasmid pTRC99a [gi:340740]. It is used for tac promoter regulated high-level expression of cloned genes such as Cytochrome P450s in <i>E. coli</i> without fusion tag sequences coded by the vector. Fusion tags are added to inserts using PCR primers. Two stop codons are included in the vector at the C-terminal cloning site.
Antibiotic resistance	Ampicillin, 100 ug/ml
Promoter	Single tac promoter from pKK233-2
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using BD-Biosciences Infusion enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	ATG in vector at 3690 bp
N – terminal fusion sequence	none
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	
5' primer for amplification of insert	5' AGG AAA CAG ACC ATG --- 3'
3' primer for amplification of insert	5' CAA GCT TCG TCA TCA --- 3'
5' sequencing primer pTRC-fwd	5' AGCTGTTGACAATTAATCATCC 3'
3' sequencing primer pTRC-rev	5' ATCAGACCGCTTCTGCGTTC 3'

## pTRC-LIC vector map

trc promoter	3441-3686
N-terminal cloning site	3678-3692
C-terminal cloning site	5708-5722
5S RNA, T1, T2, rrnB	5724-6148
<i>bla</i> coding sequence	93-950
pBR322 origin	1711
<i>lacI</i> coding sequence	2302-3381
<i>sacB</i> coding sequence	4214-5632



pTRC-LIC cloning/expression region

pTRC-fwd primer

.....  
3579 atcataacgg ttctggcaaa tattctgaaa tgagctggtg acaattaatc  
tagtattgcc aagaccgttt ataagacttt actcgacaac tgtaattag

.....▶  
3629 atccggctcg tataatgtgt ggaattgtga gcgataaca atttcacaca  
taggccgagc atattacaca ccttaacact cgcctattgt taaagtgtg

Met BseRI  
3679 ggaaacagac catg/attatg agttctcctc--SacB cassette(2 kb)-  
cctttgtctg gtac/taatac tcaagaggag

BseRI stop HindIII  
5701 gaggagatca tgcaca/tgat gacgaagctt ggctgttttg gcggatgaga  
ctcctctagt acgtgt/acta ctgcttcgaa ccgacaaaac cgcctactct

pTRC-rev primer

◀.....  
5742 gaagatthtc agcctgatac agattaaatc agaacgcaga agcggctctga  
cttctaaaag tcggactatg tctaatttag tcttgctctc tcgccagact

.....  
5792 taaaacagaa tttgctggc ggcagtagcg cggtgggtccc acctgacccc  
atthtctctt aaacggaccg ccgtcatcgc gccaccaggg tggactgggg