

p15TV-L Vector (GenBank accession EF456736)

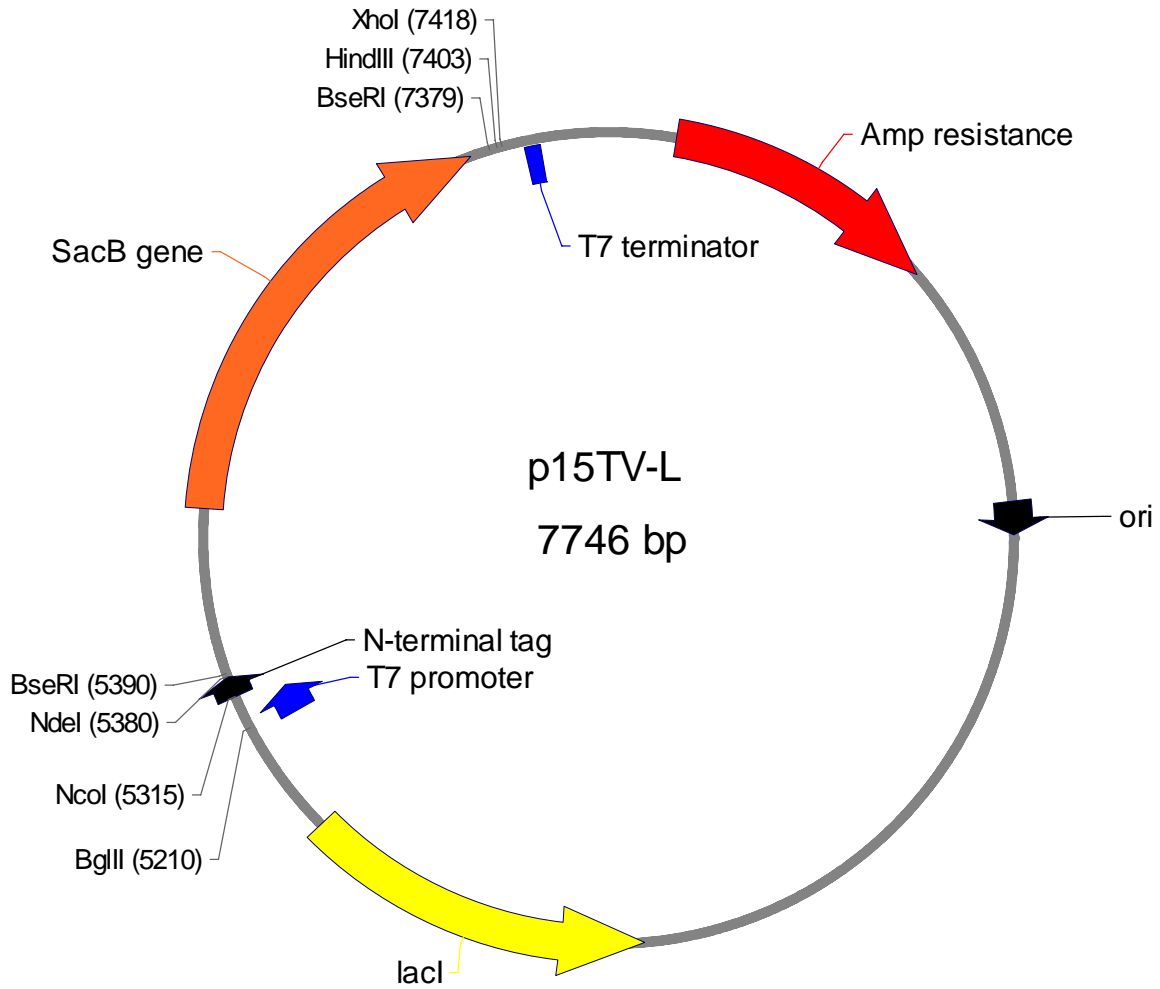
Source	Constructed by Jennifer Guthrie
Company	Clinical Genomics Centre, University Health Network, Toronto.

Description	The p15TV-L vector is a derivative of pET15b (Novagen) and is for infusion based cloning. It is used for T7 promoter driven expression of recombinant proteins with the addition of a 21 amino acid N-terminal fusion tag with 6X His followed by a TEV cleavage site. Two stop codons are included at C-terminal cloning site.
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Antibiotic resistance	Ampicillin 100 ug/ml
Promoter	T7 - lacO
Cloning Method	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	NcoI site in vector
N – terminal fusion sequence	MGSSHHHHHSSGRENLYFQG
N – terminal fusion MW	
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' TTG TAT TTC CAG GGC --- 3'
3' primer for amplification of insert	5' CAA GCT TCG TCA TCA --- 3'
5' sequencing primer T7-Fwd	5' AAT TAA TAC GAC TCA CTA TAG GG 3'
3' sequencing primer T7-Rev	5' ATG CTA GTT ATT GCT CAG CGG 3'

p15TV-L vector map

T7 promoter	5230-5246
N-terminal tag	5317-5379
N-terminal cloning site	5365-5379
C-terminal cloning site	7395-7409
T7 terminator	7488-7534
<i>bla</i> coding sequence	209-1066
pBR322 origin	1827
<i>lacI</i> coding sequence	3764-4843
<i>sacB</i> coding sequence	5901-7319



p15TV-L cloning/expression region

T7 FWD

5214 ctcgatcccc cgaaattaat acgactcact ataggggaat tgtgagcggg
gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

XbaI

5264 taacaattcc cctctagaaa taatdddgtt taactttaag aaggagatat
attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

NcoI

5314 M G S S H H H H H S S G R E N
accatgggca gcagccatca tcatcatcat cacagcagcg gcagagaaaa
tggtagccgt cgtcggtagt agtagtagta gtgtcgtcgc cgtctctttt

L Y F Q G NdeI BseRI

5364 cttgtatttc cagggc/cata tgagttctcc tc-----SACB(2 kb)---
gaacataaag gtcccg/gtat actcaagagg ag

BseRI stop HindIII XhoI

7379 gaggagatca tgcaca/tgat gacgaagctt gcggccgcac tcgaggatcc
ctcctctagt acgtgt/acta ctgcttcgaa cgccggcgtg agctcctagg

7429 ggctgctaac aaagcccgaag aggaagctga gttggctgct gccaccgctg
ccgacgattg tttcgggctt tccttcgact caaccgacga cggtaggcgac

T7 REV

7479 agcaataact agcataacc cttggggcct ctaaaccgggt cttgaggggt
tcgttattga tcgtattggg gaaccccgga gatttgcca gaactcccca