

pACYC-LIC+ Vector (not entered into GenBank)

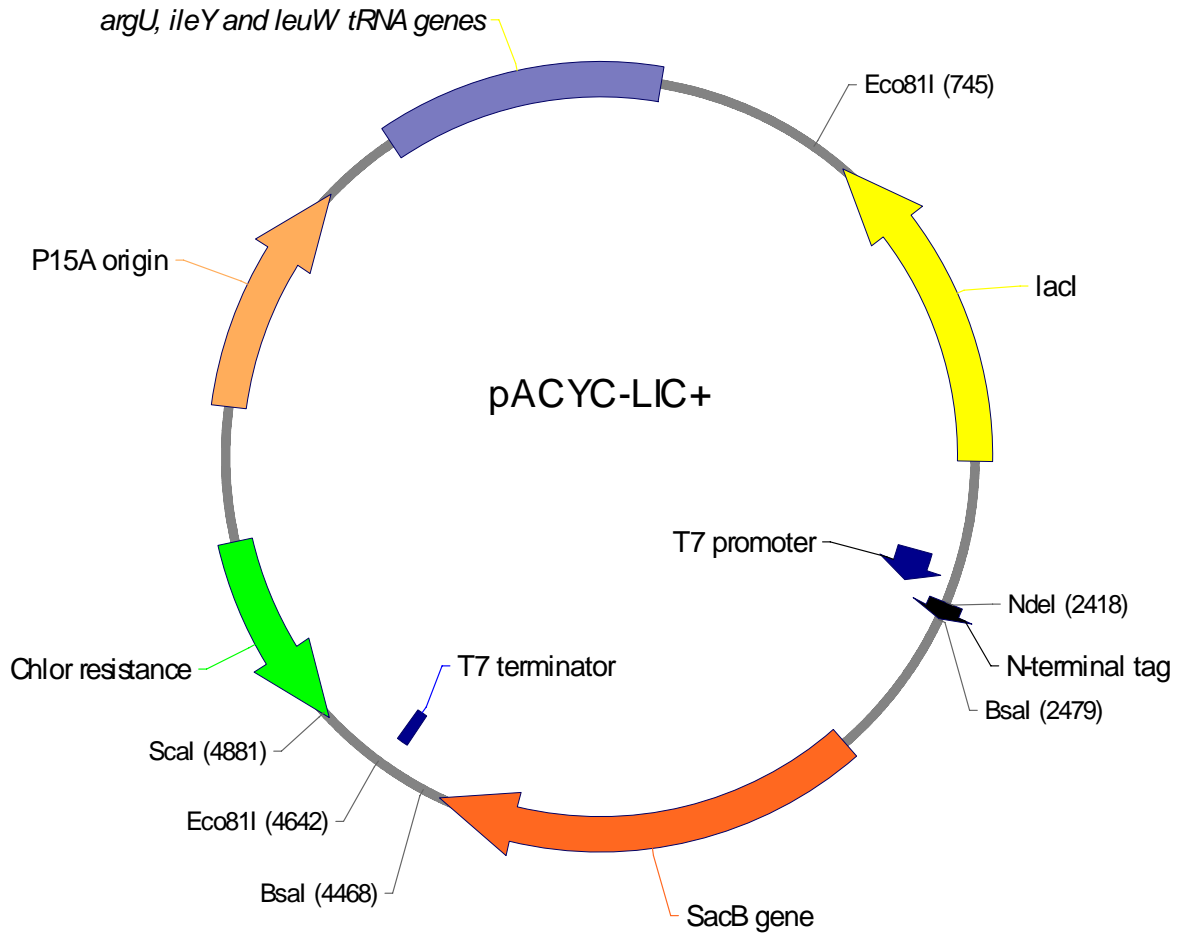
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pACYC-LIC+ vector contains the p15A origin, <i>cat</i> gene and <i>argU</i> , <i>ileY</i> and <i>leuW</i> tRNA genes of pACYC-RIL (Stratagene). It has the <i>lacI</i> , T7 promoter and T7 terminator regions from pET28a-LIC-BsaI (SGC). The plasmid is compatible with pET based plasmids. It is used for co-expression of recombinant proteins with the addition of a 19 amino acid N-terminal fusion tag containing a 6X His-tag followed by a thrombin protease cleavage site. Two stop codons are included in the vector at the C-terminal cloning site.
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Antibiotic resistance	Chloramphenicol, 25 ug/ml
Promoter	T7 - lacO
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using BD-Biosciences Infusion enzyme mediated directional recombination between complementary nucleotide DNA sequences at the ends of the insert (PCR product) and BsaI 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	NdeI site in vector at 2421 bp
N – terminal fusion sequence	MGSSHHHHHHSSGLVPRGS
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
5' primer for amplification of insert with tag	5' CCT GGT TCC GCG TGG TAG T --- 3'
5' primer for amplification of insert without tag for NdeI/BsaI cloning	5' AGA AGG AGA TAT ACA TAT G ---3'
3' primer for amplification of insert	5' GCC GCA AGC TTC GTC ATC A --- 3'
5' sequencing primer T7-Fwd	5' AATTAATACGACTCACTATAGGG 3'
3' sequencing primer T7-Rev	5' ATGCTAGTTATTGCTCAGCGG 3'

pACYC-LIC+ vector map

T7 promoter	2333-2349
N-terminal tag coding sequence	2421-2477
N-terminal cloning site	2459-2477
C-terminal cloning site	4475-4493
T7 terminator	4594-4635
<i>cat</i> (Cm ^R) coding sequence	4858-5514
P15A origin	5960-6740
<i>lacI</i> coding sequence	867-1946
<i>sacB</i> coding sequence	2990-4408



pACYC-LIC+ cloning/expression region

T7 FWD lac operator

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2317 ctcgatcccg cgaaattaat acgactcact ataggggaat tgtgagcggg
gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

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2367 taacaattcc cctctagaaa taattttggt taactttaag aaggagatat  
attgttaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

NdeI

M G S S H H H H H S S G L V P

2417 acatattgggca gcagccatca tcatcatcat cacagcagcg gcttggttcc  
tgtatacccgct cgctggtagt agtagtagta gtgtcgtcgc cggaccaagg

R G S BsaI

2468 gcgtggtagt/agagaccctg aaagatccat--SACB cassette(2 kb)--  
cgcaccatca/tctctgggac tttctaggta

BsaI stop HindIII XhoI

4459 gcacttttcg gtctcg/tgat gacgaagctt gcggccgcac tcgagcacca  
cgtgaaaagc cagagc/acta ctgcttcgaa cgccggcgtg agctcgtgga

4506 ccaccaccac cactgagatc cggctgctaa caaagcccga aaggaagctg  
gggggtgggt gtgactctag gccgacgatt gtttcgggct ttccttcgac

T7 REV

◀.....

4559 agttggctgc tgccaccgct gagcaataac tagcataacc ctttggggcc  
tcaaccgacg acggtggcga ctcgttattg atcgtattgg ggaaccccg

pACYC-LIC+ sequence

DNA sequence of plasmid beyond 6800 bp is unknown, it is not as listed below, instead it contains the tRNA genes.

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