Investigator: James DeCaprio Date: 6/1/2003 ID #:

Vector Name:	pLB(N)CX-FLAG-CH3 *

Insert

Common Name:	SV40 LT binding domain of p300 (CH3)	Gene Name:	EP300	Access. #:	U01877
Mutations:	no				
ACC# 5'-aa:	1709	ACC# 3'-aa:	1913	Organism:	*homo sapiens*
Size (bp):	690				
5'-Tag:	FLAG	3'-Tag:	no	Sequenced?	Yes
Source:	PCR product using pLB(N)CX AT-CH3-FLAG-HA				

Vector Backbone

Parental Vector:	pLB(N)CX	Type:	retrovirus	Size (kb):	6223
5'-Cloning Site:	HindIII	3'-Cloning Site:	HindIII	Promoter:	CMV
Preserved?	Yes	Preserved?	Yes		
Bacterial Selection:	ampicillin	Mammalian Selection:	blasticidin	Company:	see below
5'-Primer Name:	pLNXC F	5'-Primer Sequence:	agctcgtttagtgaaccgtcagatcg		
3'-Primer Name:	pLNCX R	3'-Primer Sequence:	acctacaggtggggtctttcattccc		

Cloning Notes:

***AKA pLB(N)CX N-FLAG hp300 LT (WT)**

A region of human p300 that spanned the SV40 LT binding domain (1709-1913 aa) (CH3 domain, described by R. Eckner, 1996) was PCR cloned using pLB(N)CX AT-CH3-FLAG-HA as the template and ligated into the pGEM-T shuttle vector. The N-FLAG CH3 insert was released using the PCR-generated HindIII restriction site and cloned into the HindIII site of pLB(N)CX. Tandem glycine residues inserted between the FLAG and p300 LT sequences were added as flexible hinges.

The N-Flag CH3 insert can also be released using 5'-BamHI and 3'-EcoRI sites that have been engineered just inside the HindIII sites.

pLB(N)CX is a derivative of Clontech retroviral pLNCX: The original pLNCX Neomycin resistance cassette was removed through 5'-B*sa*BI and 3'-B*st*BI restriction digestion and replaced with Blasticidin resistance cassette cloned in using 5'-S*ma*I and 3'-B*st*BI ends, resulting in conversion of the original pLNCX backbone sequence from 5'-GATGAGG**A**TC-3' to 5'-GATG*****GG**G**TC-3' and loss of the B*sa*BI site (***** denotes a nonconsequential loss of base during ligation). All other flanking pLNCX backbone sequences preserved.

Reference: Borger & DeCaprio (J Virol. 2006 May;80(9):4292-303)

Map: