

Date: 6/1/2003

ID #:

**pLB(N)CX-FLAG-CH3 Del33 \*****Insert**

SV40 LT binding domain of Gene Name: EP300 Access. #: U01877  
 p300 (CH3)  
 deletion of amino acids 1737-1836 (disruption of SV40 LT binding)  
ACC# 5'-aa: 1709 ACC# 3'-aa: 1913 Organism: *homo sapiens* Size (bp): 390  
5'-Tag: FLAG 3'-Tag: no Sequenced? Yes  
Source: PCR product using pLB(N)CX AT-CH3-FLAG-HA Del33

**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: agctcgttagtgaaccgtcagatcg  
3'-Primer Name: pLNXC R 3'-Primer Sequence: acctacagtggtgggtcttccattccc

**Cloning Notes: \*AKA pLB(N)CX N-FLAG hp300 LT (Del33)**

A region of human p300 that spanned the SV40 LT binding domain (1709-1913 aa) (CH3 domain, described by R. Eckner, 1996) containing a deletion of aa1737-1836 was PCR cloned using pLB(N)CX AT-CH3-FLAG-HA Del33 as the template and ligated into the pGEM-T shuttle vector. The N-FLAG CH3 Del33 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 Del33 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'-BsaBI and 3'-BstBI restriction digestion and replaced with Blasticidin resistance cassette cloned in using 5'-SmaI and 3'-BstBI ends, resulting in conversion of the original pLNCX backbone sequence from 5'-GATGAGGATC-3' to 5'-GATG\*GGGTC-3' and loss of the BsaBI 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:**