Investigator: James DeCaprio Date: 6/31/2002 ID #:

Vector Name:	**pLB(N)CX AT-CH3-FLAG-HA Del33 ***

Insert

Common Name: p300 acetyltransferase-CH3 fragment Gene Name: EP300 Access. #: U01877

Mutations: Del33 (deletion of aa 1737-1836 in CH3 domain, disrupting SV40 LT binding)

ACC# 5'-aa: 1196 ACC# 3'-aa: 1922 Organism: *homo sapiens* Size (bp): 2050

5'-Tag: no 3'-Tag: FLAG-HA Sequenced? Yes

Source: CMVbeta-p300-CHA Del33 (R Eckner)

Vector Backbone

Parental Vector: pLB(N)CX Type: retrovirus Size (kb): 6223

5'-Cloning Site: HindIII Preserved? Yes 3'-Cloning Site: HpaI Preserved? Yes Promoter: CMV

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 hp300 AT/LT C-FLAG/HA Del33**

The Del33 mutant (deletion of aa1737-1836) was produced by releasing the AT-CH3 domain from pLB(N)CX AT-CH3-FLAG-HA using the fragment internal restriction sites *Bgl*II and *Apa*I and then replacing with the *Bgl*II-*Apa*I mutant AT-CH3 fragment obtained from CMVβ-p300-CHA Del33.

This fragment is comprised of amimo acids 1196-1922 of human p300. This consists of the PHD domain, the entire acetyltransferase domain and the (mutated) adjacent SV40 LT binding domain (CH3) as described by Bordoli *et al*., *NAR* 2001 and Eckner *et al*., *MCB* 1996, respectively. An alanine residue immediately follows the initiation codon as part of the kozak sequence (italics). Tandem glycine residues inserted between the end of the p300 fragment and the start of the C-terminal tags (and also between the FLAG and HA epitopes) were added as flexible hinges. The Del33 mutation disrupts the ability of SV40 LT to bind the CH3 domain. An additional mutation in the acetyltransferase domain [y(1381)H] was identified through sequencing.

The p300 fragment alone (without stop codon) can be released by *Hin*dIII (5') and *Apa*I (3') digestion and the complete p300 fragment with C-terminal FLAG-HA tag can be released through *Hin*dIII (5') and *Hpa*I digestion.

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: