

Investigator: James DeCaprio

Date: 6/31/2002

ID #:

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

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

Mutations: no

ACC# 5'-aa: 1196      ACC# 3'-aa: 1922      Organism: *homo sapiens*      Size (bp): 2270

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

Source: PCR product using CMVbeta-p300-CHA (R Eckner)**Vector Backbone**

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

5'-Cloning Site: HindIII      3'-Cloning Site: HpaI      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: acctacagtggggtctttcattccc

**Cloning Notes:** \* AKA pLB(N)CX hp300 AT/LT C-FLAG/HA WT

A region of human p300 that spanned the entire acetyltransferase domain through the adjacent CH3 domain (1196-1922 aa) (AT-CH3) was PCR cloned using CMVβ-p300-CHA as the template and ligated in-frame into the *Hind*III and *Apa*I restriction sites of pLB(N)CX C-FLAG-HA. 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. 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 *Hind*III (5') and *Apa*I (3') digestion and the complete p300 fragment with C-terminal FLAG-HA tag can be released through *Hind*III (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'-*Bsa*BI and 3'-*Bst*BI restriction digestion and replaced with Blasticidin resistance cassette cloned in using 5'-*Sma*I and 3'-*Bst*BI ends, resulting in conversion of the original pLNCX backbone sequence from 5'-GATGAGGATC-3' to 5'-GATG\*GGGTC-3' and loss of the *Bsa*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:**