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

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

Insert

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

Mutations: FPY(1353-1355)AAA (interference of acetyltransferase function)

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

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

Source: CMVbeta-p300-CHA FPY (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 FPY**

The FPY mutant 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 FPY.

This fragment is comprised of amimo acids 1196-1922 of human p300. This consists of the PHD domain, the entire acetyltransferase domain and the 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 FPY(1353-1355)AAA mutation preserves intrinsic HAT activity but completely interferes with ability to bind and acetylate substrates such as p53 and histones (Bordoli, 2001). 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: