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Primer Design for Cloning Genes into the pHAGE Lentiviral Backbone

Positions for cloning:
1st position: promoter: spe1/Not1
gene CDS: Not1/BamH1
NB: Not1= GCGGCCGC and Kozak consensus sequence=

GCC^{A}/TGCCATGG; so a 5' oligo for PCR of a gene to clone into position one should add NNN-GCGGCCGCC in front of the ATG start codon for that gene thus adding the Kozak consensus sequence with embedded Not1 site onto the 5' end of the gene! (NNN= three random nucleotides {e.g. TTT} in front of the restriction site to allow Not1 enzyme to cut efficiently.

2nd position: promoter: BamH1/Nde1
gene CDS: Nde1/Cla1
NB: Nde1 enzyme requires 7 base pairs in after the restriction sequence for efficient cutting. Also beware CMV promoter has an internal Nde1 site so this site will not be unique when working with CMV containing constructs. Also ClaI in certain context gets dam methylated (see other protocol titled ClaI dam methylation).

How to design primers using Vector NTI:
Enter sequence of your gene. Start with the 5' (upper) primer. Paste about 40 base pairs of the 5' leading end of your sequence into the oligo list. Add the restriction site in front of your sequence with extra leading base pairs (e.g 3 extra base pairs for not1 to allow cutting; don't forget to add kozak consensus sequence in front of ATG for genes). Save this sequence in the oligo list as' oligo.

Next analyze and trim the oligo: In the oligo menu click analyze. Remove the extra (non-complementary) leading sequence that you are going to add to the 5' end of the gene as this will not bind to anything on the first PCR cycle: click analyze. Begin trimming bases away from the 3' end of the sequence until the Tm is about 60 degrees. Paste back the 5' leading sequence that you want to add to the gene during PCR. Save this and analyze it for hairpins, dimmers etc.

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