FUNCTIONAL GENOMICS FACILITY
at the University of Colorado at Boulder

In order to generate an accurate map of the pLX304 vector containing your ORF of interest, replace the sequence below highlighted in bright green (ATGNNNNNNNNNN) with the sequence of your ORF, starting with the ATG of the ORF and ending with the last codon before the native stop codon. The codon between the 3' end of your ORF and the 3' Gateway site (XXX) is one of three possible codons (TAC, TTG or TGC). If an ORF clone has been sequenced by The Broad, the identity of the variable codon can be obtained by going to http://www.broadinstitute.org/rnai/public/clone/search, searching for your clone number, clicking on the clone ID link and then looking at the Empirically Derived Sequence data, specifically at the 3' flank sequence. If a clone has not been sequenced, you will need to determine the identity of the variable codon by sequencing yourself.

pLX304 Lentiviral Expression Vector sequence containing hypothetical ORF

cccgggttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggc
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IMPORTANT SEQUENCE FEATURES -

5’ sequencing primer: 5’-cgcaaatgggcggtaggcgtg-3’

3’ sequencing primer: 5’-tacgggaagcaatagcatga-3’

5’ Gateway site: ACAAGTTTGTACAAAAAAGTTGGC

3’ Gateway site: CCAACTTTCTTGTACAAAGTGG

Your favorite ORF, beginning with the start codon and ***excluding*** the native stop codon: ATGNNNNNNNNNN

Variable codon, XXX: This codon could be one of three possible codons, TAC, TTG or TGC

V5 epitope tag sequence: ggtaagcctatccctaaccctctcctcggtctcgattctacg (translation: GKPIPNPLLGLDST)

stop codon: TAG

Document produced by Ryan Henry, Ph.D. (Last modified: 04/02/2013)