

1. LIC Primer Design Protocol

Version Number : 1

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Author: H.Yokota

Summary: LIC primers require addition of non-complementary Linker Sequences. These sequences are required to allow single-stranded ends to be made on the PCR product. These ends are designed to be complementary to the prepared single-stranded ends of our existing LIC “B” Vectors.

Materials/Reagents/Equipment	Vendor	Stock Number
Equipment		
Computer with Web linkage		
Genome Text files with DNA Sequences	Various Databases	

Procedure
Obtain the Gene DNA Sequence: BSGC Website, NCBI or PEDANT Database.
N-terminal Gene Sequence
If Initiation Codon is NOT an ATG, use only the “TG” for Melting Temperature [T _m] Determination.
Calculate T _m using website: Calculated T _m should ideally be at least _____. [Previous primer T _m s have been lower – results yielded poor PCR reactions.]
Add Linker 1 Sequence to the 5’ end: GGC GGT GGT GGC GGC (A[<u>TG</u>]), where the (A[<u>TG</u>]), is the Initiation Codon. For Gene sequences having the ATG, omit the A; for those Genes not starting with ATG, add the A. In other words, the initiation codon should be changed to “ATG”. [Note: This Linker 1 codes for 5 glycines.]
C-terminal Gene Sequence
If Termination Codon is NOT a TAG, use only the “TA” [for TAA] or “T” [for TGA]. Calculate T _m to be at least 62.0.
Add Linker 2 Sequence and Termination Sequence to the 5’ end of this sequence: <u>G TTC TTC TCC TTT GCG CCC CTA</u> , where the CTA is the Termination Codon. For sequences already having the “A” only add the “CT” and for sequences already having the “TA” only add the “C”. [This Linker 2 codes for the N-terminus of modified GFP.]
Obtain the Reverse Complement using the website: http://arbl.cvmbs.colostate.edu/molkit/manip/

1b. Primer Ordering

Currently using IDT for oligonucleotide ordering.