1. LIC Primer Design Protocol
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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 [Tm] Determination.
Calculate Tm using website: Calculated Tm should ideally be at least ___. [Previous primer Tms have been lower – results yielded poor PCR reactions.]
Add Linker 1 Sequence to the 5' end: GGC GGT GGT GGC GGC (A[TG]), where the (A[TG]), 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 Tm to be at least 62.0.
Add Linker 2 Sequence and Termination Sequence to the 5' end of this sequence: G TTC TTC TCC TTT GCG CCC CTA, 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.