Lab 1: part 4: M13K07 tag

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*useful info: BamHI cut site: 5' GGATCC 3' ----> 5' G GATCC 3'
3' CCTAGG 5' ----> 3' CCTAG G 5'

TOP STRAND:

Step 1:
Reverse translate myc epitope EQKLISEEDL:

GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG

Step 2:
Add BamHI tail to top strand so it can anneal with overhang on M13 plasmid:

5'- (GAT C)_ _GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG -3'

*note: will need to add 2 spacer bps to keep ORF same. The first spacer from 5' can not be a C or will regenerate a BamHI cut site.

Step 3:
Keep the ORF the same by adding TC in the 2 spacer positions. This adds to the p3 protein a new but (hopefully) neutral Leucine codon:

5'- (GAT C)**TC** GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG -3'

Step 4:
Create a unique EcoRV cut site in epitope sequence by changing underlined C to an A:

5'- (GAT C)**TC** GAA CAG AAA CTG ATA TCT GAA GAA GAC CTG -3'

BOTTOM STRAND:

Step 1 / Step 2:
Compliment the portion of top strand we want double stranded in final insert (i.e. ignore top strand's tail). Then add BamHI tail to bottom strand so it can anneal with overhang on M13 plasmid:

3'- **AG** CTT GTC TTT GAC TAT AGA CTT CTT CTG GA**C** (CTA G) -5'

*note: ORF is fine, but will need to change the 5th codon from 5' end to something other than a **C** b/c we have generated a BamHI site.

Step 4:

Destroy BamHI site. The complimentary portion of bottom strand 3'- GA**C** -5' is 5'- CT**G** -3'. This is a Leucine codon. Changing top strand's **G** to a **T** does not, however, alter the translational product. Adjust both strands accordingly:

Top: 5'- (GAT C)**TC** GAA CAG AAA CTG ATA TCT GAA GAA GAC CT**T**

Bottom: 3'- **AG** CTT GTC TTT GAC TAT AGA CTT CTT CTG GA**A** (CTA G)

Final Product:

*With unique EcoRV cut site underlined **GAT|ATC** and single-stranded portions in lower case:

Top strand:

5'- gat cTC GAA CAG AAA CTG ATA TCT GAA GAA GAC CTT -3'

Bottom strand:

3'- AG CTT GTC TTT GAC TAT AGA CTT CTT CTG GAA ctag -5'