Part 2 of your portfolio:

1. Choose a section (site-site)
2. Design DNA for your section (must include terminal RE sites)
3. Annotate sequence (follow scheme)
4. Write summary paragraph (include $ estimate and GO/NO-GO)
5. 4-8 week fab time, f(L, complexity)
M13K07 Segment: Wild Type

\((1-2653)\)
Idea #1 -- Get rid of protein X

Work between the MluI and Bsu36I sites
Step 3: identify flanking restriction sites

An M13K07mut8 scaffold has been made in the Endy lab to design these changes; Felix Moser, a technician in the Endy lab, designed DNA synthesis plus a little site directed mutagenesis. The

251. TGACCTCTTTA TCAAAAGGAG CAATTAAGG TACTCTCTAA TCCTGACCTG
301. TTGGAGTTTTG TTCCCGGTCT GTTTCGCTTT GAAGCTCGAA TTAAA[ACGCG
351. TTTATTGAAG TTTTTCCGGGC TTCCCCTTTAA TCTTTTTATGGT GCAATCCGCT
400. TTGGTTCTGA CTTTATAGT CAGGGTAAG ACGGATTTT TGATTTATGG
451. TGATTCTCGT TTTCTGA ACT GTTTAAAGCA TTGGAGGAGG ATTCGATGAA
501. TATTATGAC GATCCCGCAG TATGGACGC TATCCAGTCT AAACATTTTA
551. CTATTACC[C CTCAGG]CAA ACTTCTTTTG CAAAAGCCTC TCGCTATT
Find some parts. We know that gX should be somewhere around here, and maybe an RBS.
Mlu I  
[ACGCG T]TATTGGAAG TCTTTCGGGC TTCCCTTTAA TCTTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA

TATTATGAC GATTCCGCAG TATGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I  
CTATTACCC[C CTCAGG]

Option 1. Eliminate start codon

Issue: Remember pII, does it need a met?
What would be a neutral substitution for methionine?

Do a web search on "methionine substitution neutral"
[Image of Google search result removed due to copyright restrictions.]

Let's check this out...
### Lots of choices for leucine...  
### Pick a reasonable codon...  

Cite as: Drew Endy. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DB Month YYYY].
Given that we could use one of six codons for leucine, which should we choose?

Since DNA will be used in E. coli, choose a codon that E. coli likes.

Web search on "codon usage coli"
[image of Google results page removed due to copyright restrictions]
Let’s go with CUG

Mlu I

[ACGCG T]TATTTGAAG TCTTTTCGGGC TTCTTCTTTAA TCTTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCA

TTATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I

CTATTACCC[C CTCAGG]
Mlu I
[ACGCG T]TATTTGAAG TCTTTTCGGGC TTCTCTTTAA TCTTTTTTGAT GCAATCCGCT
TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG
TCATTCTCGT TTTCTGAACT GTTTAAAGC A TTTGAGGGGG ATTC AATGAA
TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
Bsu36 I
CTATTACCC[C CTCAGG]

Option 2. Trash the RBS...