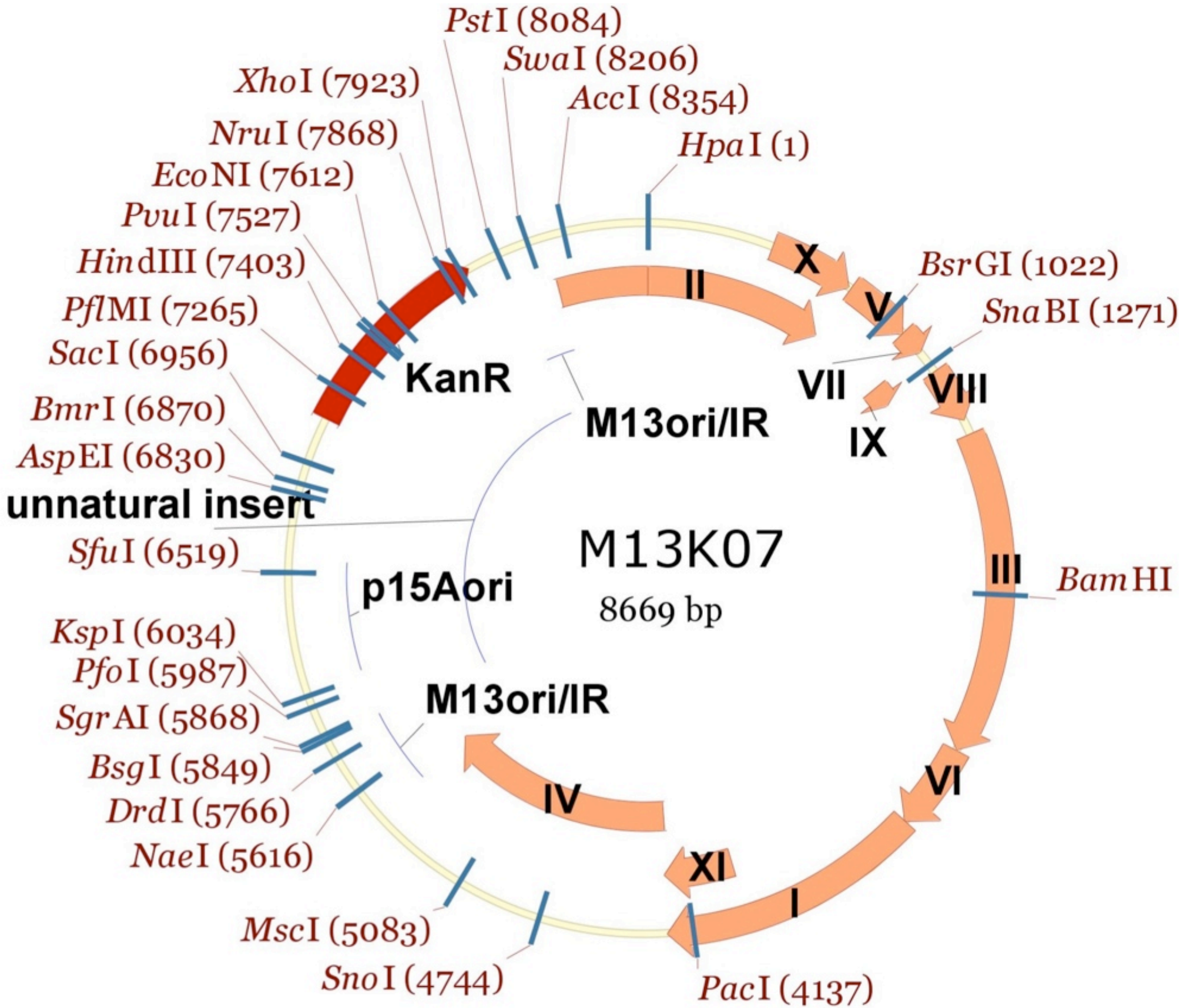
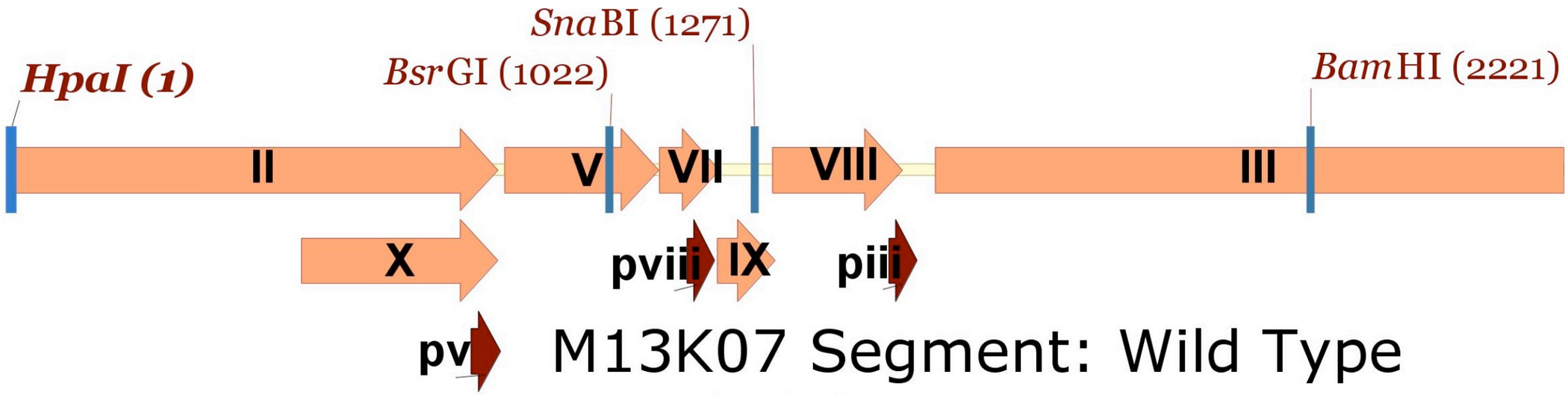
Part 2 of your portfolio:

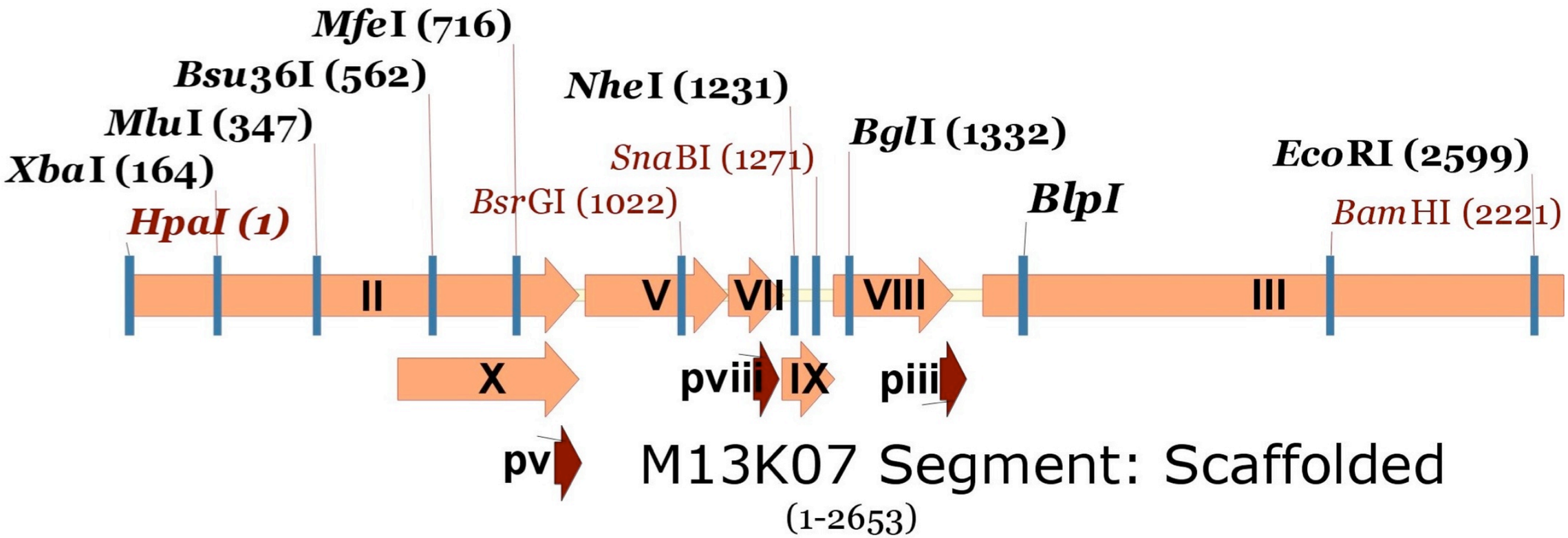
1. Choose a section (site-site)
2. Design DNA for your section (must include terminal RE sites)
3. Annotate sequence (follow scheme)
4. Write summary paragraph (include $ estimate and GO/NO-GO)
5. 4-8 week fab time, f(L, complexity)





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 t
design these changes; Felix Moser, a technician in the En
DNA synthesis plus a little site directed mutagenesis. The

```
251  TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
                                                    MluI
301  TTGGAGTTTG CTTCCGGTCT GGTTCGCTTT GAAGCTCGAA TTAAA[ACGCG

351  I]TATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT

401  TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

451  TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA

501  TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
                   Bsu36I
551  CTATTACCC[C CTCAGG]CAAA ACTTCTTTTG CAAAAGCCTC TCGCTATTTT
```

Mlu I
[ACGCG **T**]TATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA

TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I
CTATTACCC[C CTC**A**GG]

Find some parts. We know that gX should be somewhere around here, and maybe an RBS.

Mlu I
[ACGCG **T**]TATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

pX RBS pX start

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAA**ATG**AA

TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I
CTATTACCC[C CTCAGG]

Option 1. Eliminate start codon

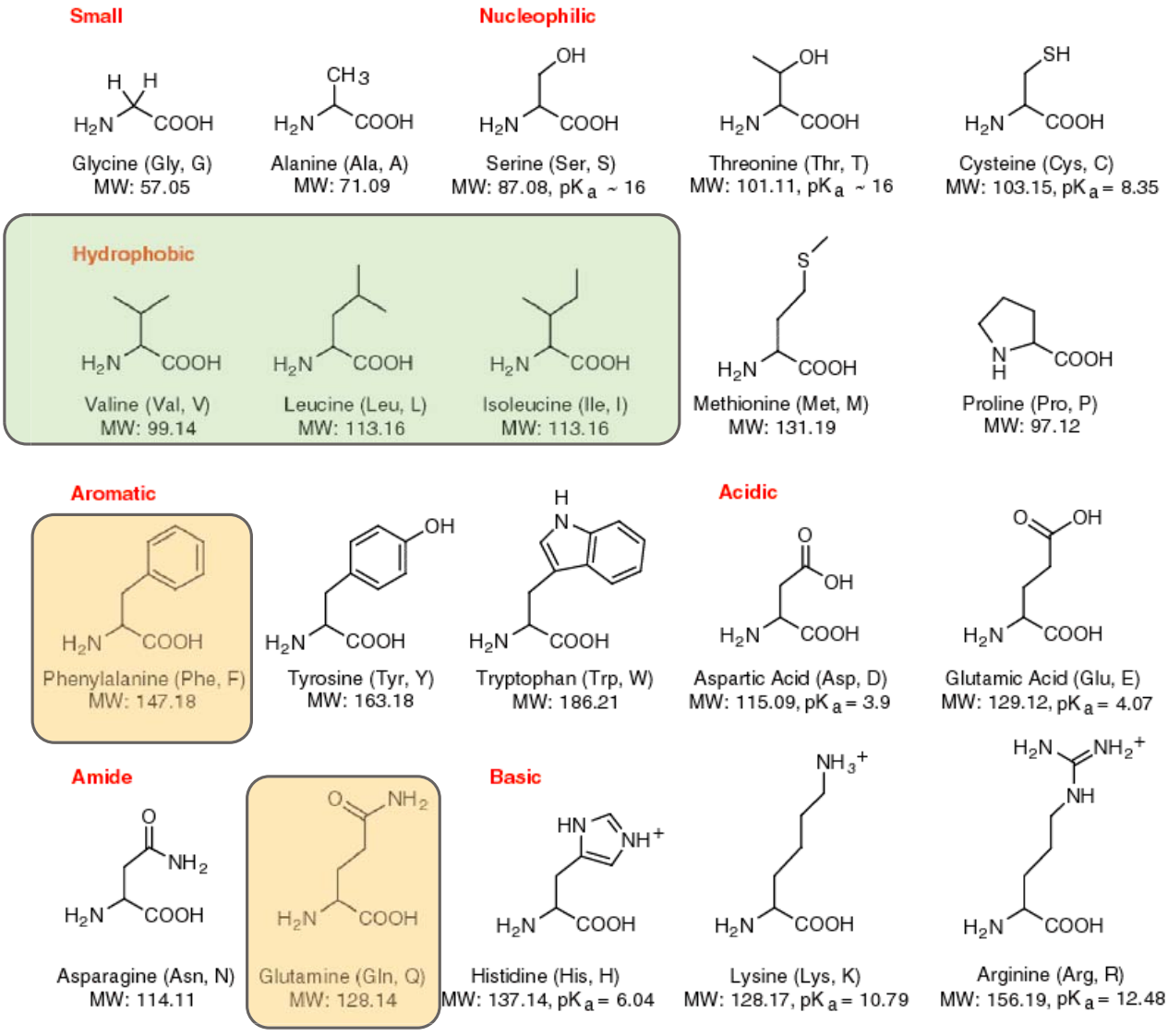
Issue: Remember pII, does it need a met?

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...



First Position (5' end)	Second Position: U		Second Position: C		Second Position: A		Second Position: G		Third Position (3' end)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	*Stop*	UGA	*Stop*	A
	UUG		UCG		UAG	*Stop*	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Figure by MIT OpenCourseWare.

Lots of choices for leucine...

Pick a reasonable codon...

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 TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

pX RBS pX start

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCA**CTG**AA

TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I
CTATTACCC[C CTC**A**GG]

Mlu I
[ACGCG T]TATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT

TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG

pX RBS pX start

TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA

TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA

Bsu36 I
CTATTACCC[C CTCAGG]

Option 2. Trash the RBS...