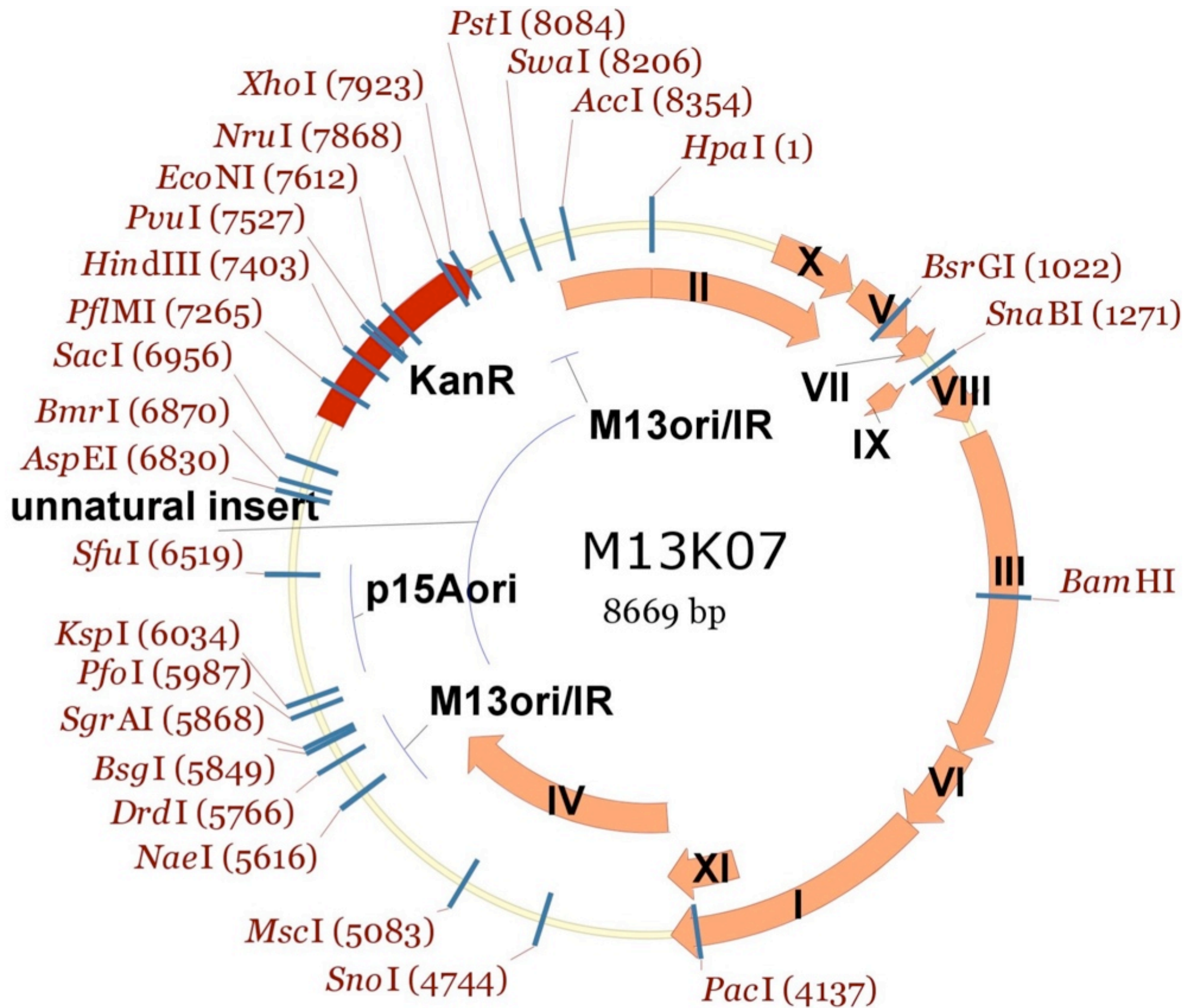
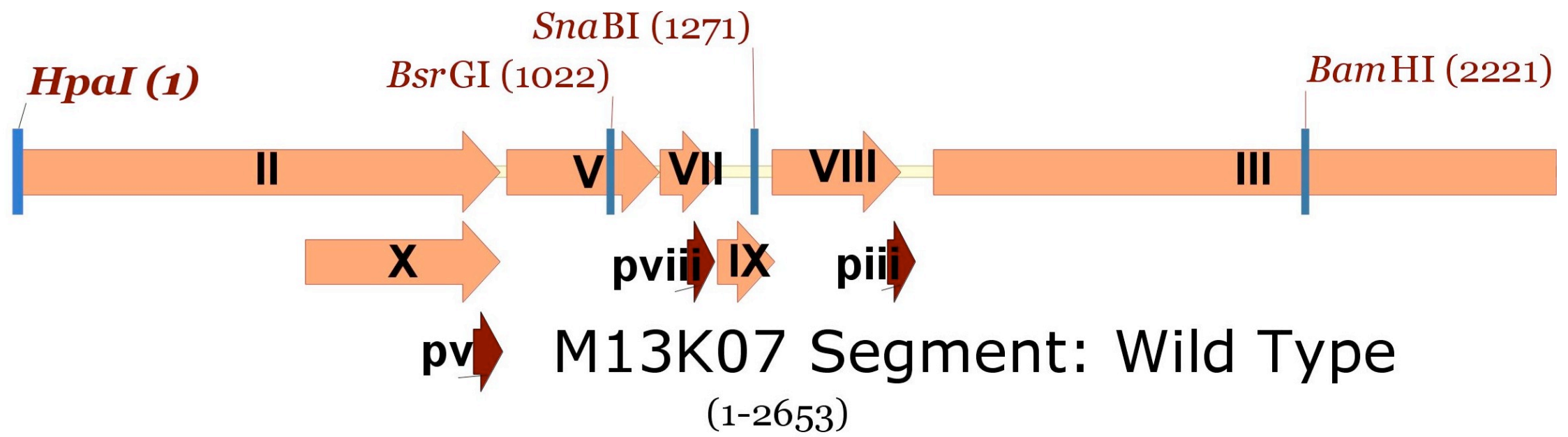
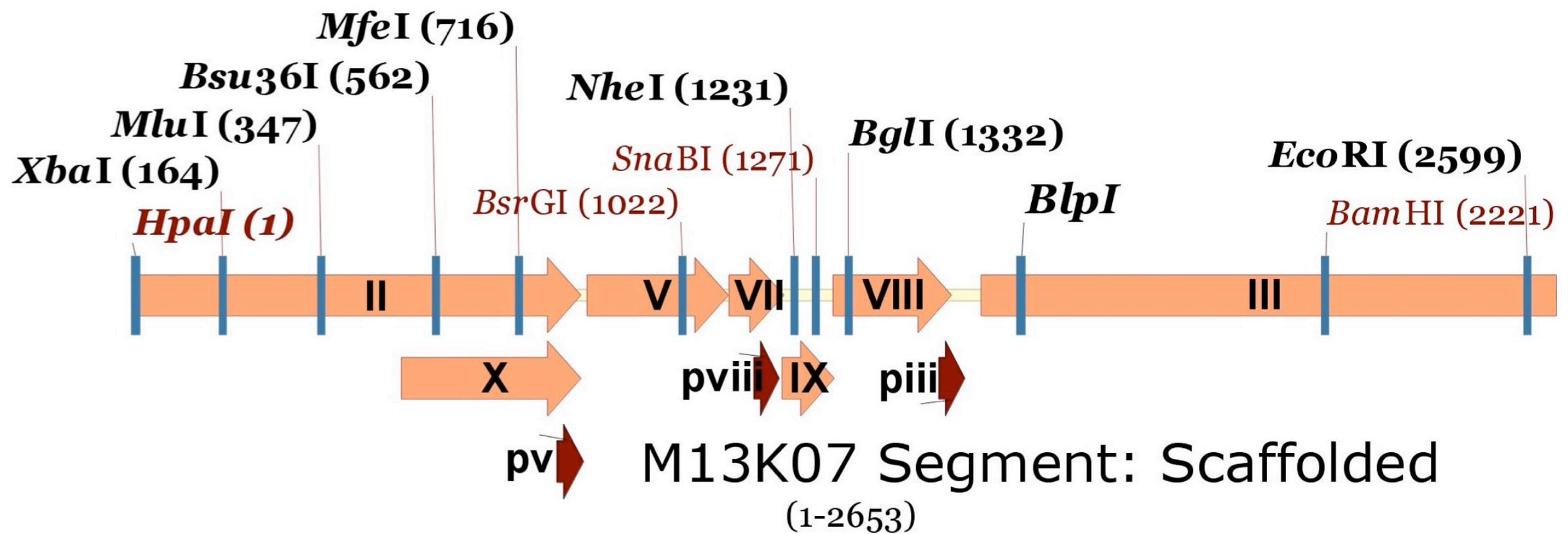


Part 2 of your portfolio:

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, $f(L, \text{complexity})$







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, performed the DNA synthesis plus a little site directed mutagenesis. The i

```
251 TGACCTCTTA TCAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
301 TTGGAGTTTG CTTCCGGTCT GGTTCCGCTTT GAAGCTCGAA TTAAA[ACGCC
351 TTTATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT
401 TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG
451 TCATTCTCGT TTTCTGAACT GTTTAAAGCA TTTGAGGGGG ATTCAATGAA
501 TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
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
 TCATTCTCGT TTTCTGAACT GTTTAAAGCA **TTTGAGGGGG** **ATTCAATGAA**
 TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
 Bsu36 I
 CTATTACCC[C CTC**A**GG]

Option 1. Eliminate start codon

Issue: Remember pII, does it need a met?



methionine substitution neutral

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[\[PDF\] Effect of Threonine-to-Methionine Substitution Structure and ...](#)

Effect of Threonine-to-Methionine Substitution. at. Position 265 be neutral with. respect to. catalysis and. enzyme expression. MATERIALS ...

aac.asm.org/cgi/reprint/38/10/2266.pdf - [Similar pages](#) - [Note this](#)

[Mutational analysis of methionine adenosyltransferase from ...](#)

Site-directed mutagenesis was used to substitute neutral residues for the substitution, site-directed mutagenesis of lysine 165 to methionine in E. ...

content.febsjournal.org/cgi/content/full/271/13/2791 - [Similar pages](#) - [Note this](#)

[Covalent intermediate trapped in 2-keto-3-deoxy-6 ...](#)

Hydrolysis transforms the Schiff base into a neutral carbinolamine species because of seleno-methionine substitution that perturbed trimer packing. ...

www.pubmedcentral.nih.gov/articlerender.fcgi?artid=31111 - [Similar pages](#) - [Note this](#)

[Methionine](#)

Substitutions: As Methionine is a hydrophobic amino acid, and can nearly be classed with other aliphatic amino acids. It prefers substitution with other ...

www.russell.embl-heidelberg.de/aas/Met.html - 13k - [Cached](#) - [Similar pages](#) - [Note this](#)

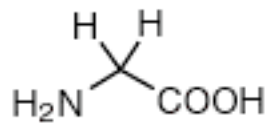
[\[PDF\] SUBSTITUTION OF DL-METHIONINE FOR SOYBEAN MEAL AS A WINTER ...](#)

Neutral detergent fiber. also was determined (Van Soest and Robert- DL-methionine in a beet pulp carrier did not. substitute for soybean meal as a ...

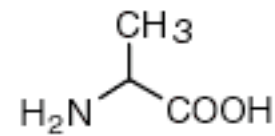
jas.fass.org/cgi/reprint/68/12/4361.pdf - [Similar pages](#) - [Note this](#)

Let's check this out...

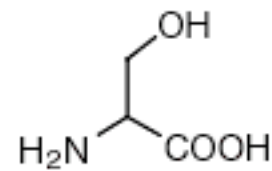
Small



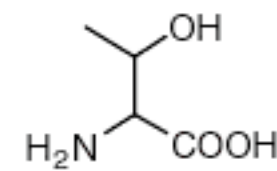
Glycine (Gly, G)
MW: 57.05



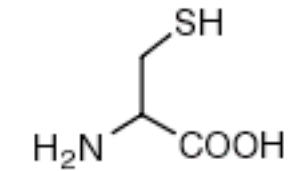
Alanine (Ala, A)
MW: 71.09



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

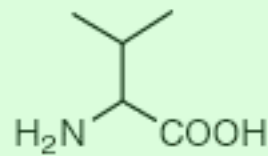


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

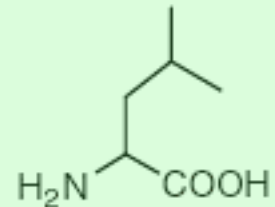


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

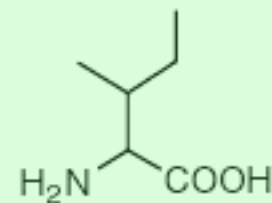
Hydrophobic



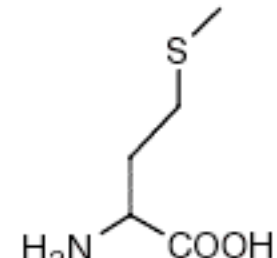
Valine (Val, V)
MW: 99.14



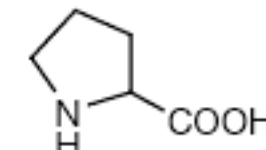
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

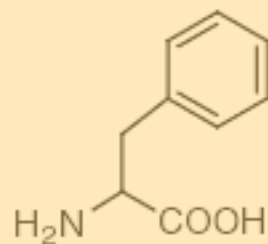


Methionine (Met, M)
MW: 131.19

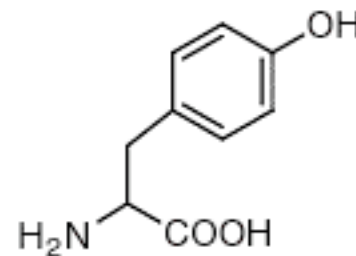


Proline (Pro, P)
MW: 97.12

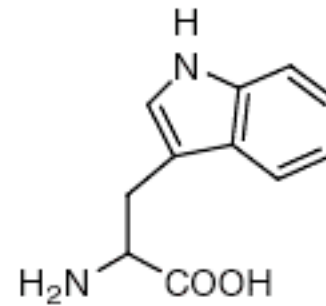
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

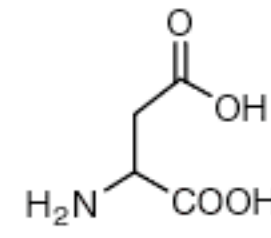


Tyrosine (Tyr, Y)
MW: 163.18

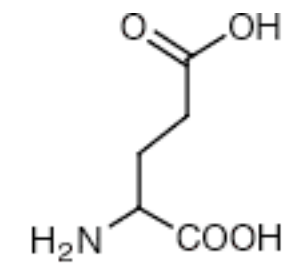


Tryptophan (Trp, W)
MW: 186.21

Acidic

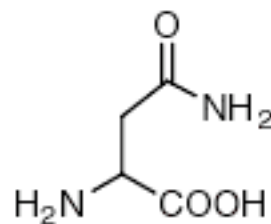


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

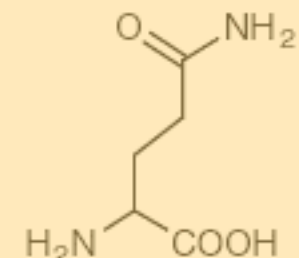


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

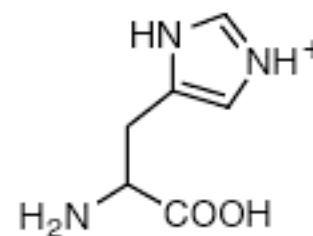


Asparagine (Asn, N)
MW: 114.11

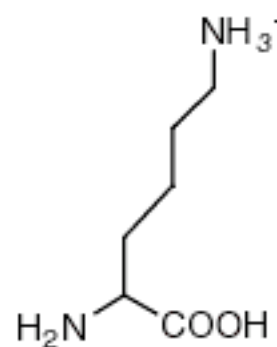


Glutamine (Gln, Q)
MW: 128.14

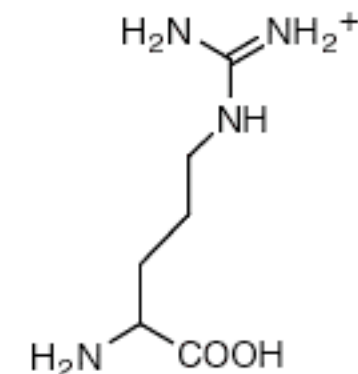
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

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

Lots of choices for leucine...
Pick a reasonable codon...



codon usage coli

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[E. coli Codon Usage](#)

Codon Usage in *E. coli* from a reference by HÉNAUT and DANCHIN. Amino Acid, **Codon**, Class, Amino Acid, **Codon**, Class. I, II, III, I, II, III ...

psyche.uthct.edu/shaun/SBlack/codonuse.html - 11k - [Cached](#) - [Similar pages](#) - [Note this](#)

[Codon usage table](#)

Escherichia **coli** [gbtct]: 14553 CDS's (4339817 codons) ... List of **codon usage** for each CDS [CAUTION: The file is big (> 1000 entries).] (format) ...

www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Escherichia+coli+%5Bgbtct%5D - 5k - [Cached](#) - [Similar pages](#) - [Note this](#)

[E. coli codon usage analyzer](#)

This Javascript will take a DNA coding sequence and display a graphic report showing the frequency with which each **codon** is used in *E. coli*. ...

www.faculty.ucr.edu/~mmaduro/codonusage/usage.htm - 12k -

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[E. coli Codon Usage](#)

Codon Usage in *E. coli* from HÉNAUT and DANCHIN: Analysis and Predictions from *Escherichia coli* sequences. *Escherichia coli* and *Salmonella*, Vol. ...

www.faculty.ucr.edu/~mmaduro/codonusage/codontable.htm - 11k -

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[e coli codon usage table](#)

www.molbiol.ox.ac.uk/~cocallag/refdata_html/codonusagetable.shtml -

[Similar pages](#) - [Note this](#)

Let's go with CUG

Mlu I

[ACGCG **T**]TATTTGAAG TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT
TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTTT TGATTTATGG
TCATTCTCGT TTTCTGAACT GTTTAAAGCA **TTTGAGGGGG** **ATTCACTGAA**
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
 TCATTCTCGT TTTCTGAACT GTTTAAAGCA **TTTGAGGGGG** **ATTCAATGAA**
 TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT AAACATTTTA
 Bsu36 I
 CTATTACCC[C CTC**A**GG]

Option 2. Trash the RBS...