Mutations Investigation Results

Use the **Sequence Results document** to choose two samples to work with.

Which samples should I choose?

- Choose one sample WITH a mutation in the LacZ region. In other words, this sample should be a mutation within the range 216-539.
- Choose one sample WITHOUT a mutation in the LacZ region. This sample should not have any mutations within the range 216-539.
- If possible, choose YOUR OWN sample to analyze.

Example:

Mutation within 216-539

Aa-White-1

CHROM	POS	REF	ALT	QUAL
pUC19	318	T	C	19542

No mutation within 216-539

Aa-Blue-4

CHROM	POS	REF	ALT	QUAL
pUC19	198	T	C	46252

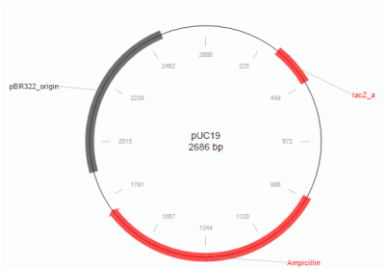
For each sample, record the following information:

- Name of the sample
- Blue or white phenotype of the sample (if known)
- What mutations occurred in this sample? Use the plasmid map (below) to identify what regions of interest were affected?

Sample WITH mutation in the LacZ region:

Sample WITHOUT mutation in the LacZ region:

Regions of interest in the pUC19 plasmid



Region	Range
LacZ alpha peptide	216-539
Lac operator (repressor binding site)	175-198
LacZ promoter	142-171
Ampicillin cassette	885-1745
Origin of replication	1900-2519

Practice using BLAST to identify the LacZ-alpha peptide

1. Go to the Mutations Investigation website: http://ase.tufts.edu/chemistry/walt/sepa/MIEresources.html
2. Download the pUC19 Reference Genome.
3. Open it using Notepad or TextEdit (or other text editor).
4. Go to the Expasy translation site: http://web.expasy.org/translate/
5. Copy paste the plasmid reference sequence into the textbox and click TRANSLATE SEQUENCE.
6. Results will appear, showing all open reading frames (ORFs) for the pUC19 plasmid.
7. Find the second longest ORF in 5'3' Frame 3. This sequence should correspond to the LacZ-alpha gene. Select and copy this ORF.
8. Go to the protein BLAST website: http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?PAGE=Proteins
9. Paste the LacZ peptide sequence into the textbox and click BLAST.
10. If you have the correct sequence, the BLAST should report a very high match for the LacZ protein. (If BLAST does not find a high match for the LacZ gene, then something is wrong and you probably have the wrong sequence. Go back to Step 7 and try again.)
11. Once you confirm the LacZ-alpha peptide using BLAST, record its amino acid sequence here (use single-letter amino acid abbreviations):

Obtain the mutated LacZ amino acid sequence

1. Open the pUC19 Reference Genome file using Notepad or other text editor.
2. For the sample containing mutations in the LacZ gene: use the listed mutations to make changes to the reference genome file.
3. Go to the Expasy *in silico* translation site: http://web.expasy.org/translate/
4. Copy the altered reference sequence into the textbox and click TRANSLATE SEQUENCE
5. Find the ORF corresponding to the LacZ peptide.
6. Record the mutated LacZ peptide sequence here:

Conclusion

Describe the changes, if any, between the two peptide sequences.

For the sample you just analyzed, would you expect the mutation in the LacZ-alpha gene to have any effect on the phenotype of an *E. coli* colony carrying this plasmid? Why or why not?