Im9 Mutants I

Purpose:
To explore protein structure and function by making novel mutations in a protein with known structure.

First: Notebook Review
To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- The function of the Im9 immunity protein and the E9 toxin.
- How to 'read' amino acid structures to determine their:
  - Shape and size
  - Ability to form ionic bonds
  - Ability to
- How the features of amino acids can influence protein structure

You will then discuss these with your TA as a class to clarify any issues that remain.

Procedure
Today, you will use your knowledge of protein structure and function to explore the Im9 protein. This protein, when functional, binds to the protein toxin Colicin E9 and prevents the toxin from killing an *E. coli* cell.

1) Each group will work together to select two mutants of the Im9 protein that we will test for function in a future lab session. One of these mutants should be chosen so that it will likely cause the altered Im9 protein to be non-functional; the other should be chosen so that the altered Im9 protein will likely be functional. There is a short video on the OLLM showing how to use the Im9 website.

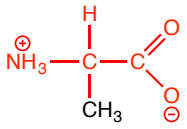
2) You should then fill in questions (1) and (2) on the worksheet at the end of this section of the lab manual.

3) You will then, as a class, decide which of the mutations the groups have proposed will be the two that will actually be tested. You should fill in questions (3) and (4) on the worksheet with this information. **Do not fill out questions (5) and (6) yet; you will answer them in a later lab.**

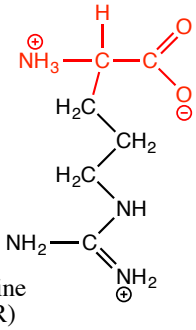
4) Between now and the next lab, BW and the course staff will grow the bacteria you have chosen, along with some control strains, on Petri dishes. You will then spread these bacteria on a plate and your TA will spot on a small spot of Colicin E9 toxin. You will then be able to see if your predictions are correct or not.

A table of amino acid structures can be found on the next page.

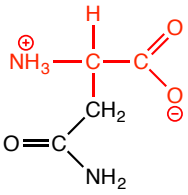
Structures of Amino Acids



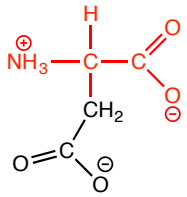
Alanine
(Ala A)



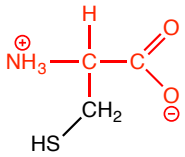
Arginine
(Arg R)



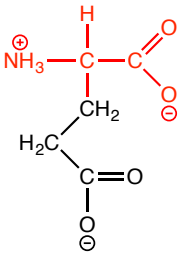
Asparagine
(Asn N)



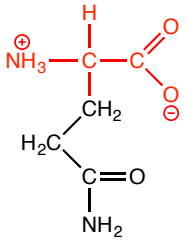
Aspartic Acid
(Asp D)



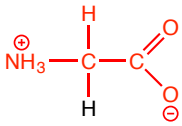
Cysteine
(Cys C)



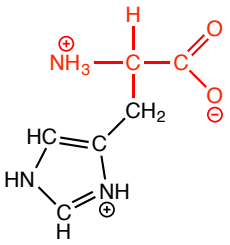
Glutamic Acid
(Glu E)



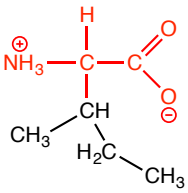
Glutamine
(Gln Q)



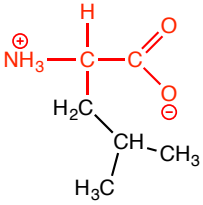
Glycine (Gly)



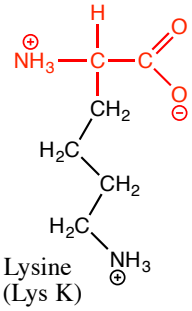
Histidine
(His H)



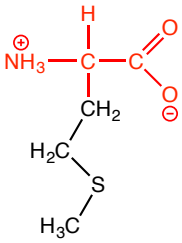
Isoleucine
(Ile I)



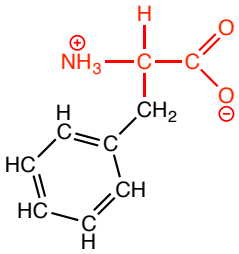
Leucine
(Leu L)



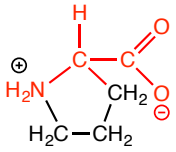
Lysine
(Lys K)



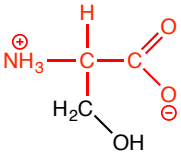
Methionine
(Met M)



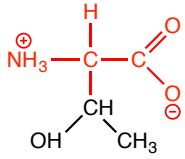
Phenylalanine
(Phe F)



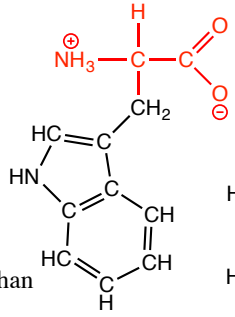
Proline
(Pro P)



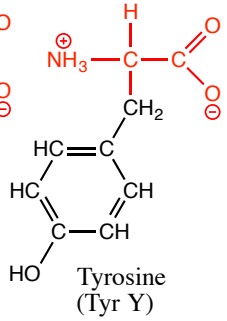
Serine
(Ser S)



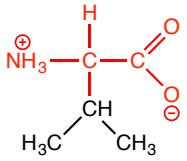
Threonine
(Thr T)



Tryptophan
(Trp W)



Tyrosine
(Tyr Y)



Valine
(Val V)

Bio 111 Im9 Mutants Worksheet

Name_____ This is a group effort for a group grade. TA & Sect._____

Name_____

Name_____ Score_____/30

<u>This is due at the end of lab today and will be returned to you in a future lab session for completion.</u>

Part I: Your <u>Group's</u> Chosen Mutations

1) Consider the mutation you have chosen that you think will result in a <u>non-functional</u> Im9 protein. You would expect cells producing this protein <u>will be killed</u> by Colicin E9 toxin. (5 pts)

a) Which amino acid do you want to change? Number_____ Amino Acid_____

b) What do you want to change it to (or delete it)? _____

c) Why do you expect this change to result in a non-functional Im9 protein?

2) Consider the mutation you have chosen that you think will result in a <u>fully-functional</u> Im9 protein. You would expect cells producing this protein <u>will not be killed</u> by Colicin E9 toxin. (5 pts)

a) Which amino acid do you want to change? Number_____ Amino Acid_____

b) What do you want to change it to (or delete it)? _____

c) Why do you expect this change to result in a fully-functional Im9 protein?

Part II: Mutations Chosen by your lab session time

After the lab sessions that meet at the same time as yours have met and chosen their two mutations, you should complete this page.

3) Consider the mutation that you and your colleagues think will result in a non-functional Im9 protein. You would expect cells producing this protein will be killed by Colicin E9 toxin. (5 pts)

a) Which amino acid do you want to change? Number_____ Amino Acid_____

b) What do you want to change it to (or delete it)? _____

c) Why do you expect this change to result in a non-functional Im9 protein?

4) Consider the mutation that you and your colleagues think will result in a fully-functional Im9 protein. You would expect cells producing this protein will not be killed by Colicin E9 toxin. (5 pts)

a) Which amino acid do you want to change? Number_____ Amino Acid_____

b) What do you want to change it to (or delete it)? _____

c) Why do you expect this change to result in a fully-functional Im9 protein?

You should stop here and turn this worksheet in to your TA.

Part III: Results (save this for a later session)

5) Consider the mutation that you and your colleagues thought would result in a non-functional Im9 protein. You would expect cells producing this protein will be killed by Colicin E9 toxin. (5 pts)

a) Which amino acid was changed? Number_____ Amino Acid_____

b) What was it changed to (or delete it)? _____

c) Were these cells killed by Colicin E9 toxin? _____

d) If the results do not agree with your prediction, provide a plausible explanation for this result.

6) Consider the mutation that you and your colleagues thought would result in a fully-functional Im9 protein. You would expect cells producing this protein will not be killed by Colicin E9 toxin. (5 pts)

a) Which amino acid was changed? Number_____ Amino Acid_____

b) What was it changed to (or delete it)? _____

c) Were these cells killed by Colicin E9 toxin? _____

d) If the results do not agree with your prediction, provide a plausible explanation for this result.