***Biol 322 Fall 2012***

***Analysis of rpoB sequence in rifamycin-resistant strains of E. coli***

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***DUE: Finals Week – no later than Thursday at 5pm***

- *This analysis is worth 35 pts*
- ***Each student must submit a hard copy of this assignment.***
- *Don't forget to email CT a digital copy of the "notes" you took as part of this online exercise.*

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***PART 1: Organize your data & online analysis and present it in table form. Also include a paragraph summarizing your findings.*** Although you are encouraged in general to organize the data as you see fit, it would be best if you organize your table by position of mutation and not by sequence number – especially since you are likely to have strains with the same mutation. See this example from last year.

| Bp Mutation | Strains w/ Mutation | DNA Mutation | EMS consistent? | Protein Mutation | AA change | AA on RpoB | Location | Codon Change | Chemical AA Change | Interact w/ Rif |
|---|---|---|---|---|---|---|---|---|---|---|
| G196A | 1E, 3E, 23E | Transition | Yes, transition | Missense | D66N | 535 | I | GAC-->AAC | Acidic to Polar | Not directly |
| C242T | 4S, 11E | Transition | Yes | Missense | S81F | 550 | | | | |

***For each rifS and rifR strain sequenced:***

1. Indicate the specific base pair mutation* (or mutations if more than one if found) and its location** using standard notation: for example, G110T indicates a G→T tranversion mutation at nucleotide position 110. What is the type of mutation at the DNA level? For terminology consult mutation jargon on the next page.
   **Since the E. coli B rpoB sequence is our reference wild-type sequence, any change from this sequence is by definition a "mutation" even if it is in a rifS strain. Focus on the sequence intervals designated in the Mutagenesis Part 4 handout.*
   *** indicate NT location relative to full-length PCR product sequence*
2. For each sequence indicate whether the wild-type or mutant line was isolated from an EMS-treated or an untreated culture. Find out about EMS-induced mutations. Is the mutation consistent with an EMS-induced change? Indicate YES or NO in your table.
3. Indicate the amino acid change and location*** using standard notation: for example, Q513L indicates a missense mutation at codon 513 where glutamine is replaced by leucine. What is the type of mutation at the protein level? Is it a chemically conservative change? What is the codon change? Did you detect any silent mutations?
   **** indicate AA location relative to PCR product sequence and to the full length protein*
4. Consult the figure showing rifR regions of the RNAP beta subunit. Is the mutation at a previously documented location? If yes, indicate the location.

http://fire.biol.wwu.edu/trent/trent/rpoBrifRregion.htm

5. Does the aa residue conferring rifR interact directly with rifamycin?
6. If you could not find a mutation in the *rpoB* region that we focused on for a particular rifR mutant indicate that the resistance ***mutation was not detected.*** Then, in your paragraph summary speculate as to why you were not able to identify the base pair change conferring resistance – there are a couple of different possible explanations here.

***Part 2: Other questions to address***

***Bacterial RNA polymerase (RNAP) is a proven target for broad-spectrum antibacterial therapy and for antituberculosis therapy. It is is a suitable target for three reasons:***
(1) RNAP is an essential enzyme *(permits efficacy)*
(2) bacterial RNAP-subunit sequences are highly conserved *(permits broad-spectrum activity)*
(3) bacterial RNAP sequences and eukaryotic RNAP sequences are less highly conserved *(facilitates therapeutic selectivity*

1. Examine the figure showing the rifR regions of the RNAP beta subunit. Which aspects of the figure document points 2&3 above?
2. The RNA polymerase in *Mycobacterium tuberculosis* is 10X more sensitive to rifamycin than the *E. coli* polymerase. How can this observation be reconciled with the striking sequence conservation shown in this figure?