

Fall 2012 Biol 322

An investigation of mutagenesis in *E. coli*

Data Work-up (Results) & Discussion (15 points)

Due on Tuesday 11/13 at the beginning of lab. No late submissions will be accepted

Have a look at this assignment before the next lab period:

On Thursday 11/8 we can discuss questions 1-4 under discussion

Class data will be posted: pool all data for your report

Spontaneous $lac^+ \rightarrow lac^-$

_____ white colonies among _____ colonies screened

Spontaneous $rif^S \rightarrow rif^R$

_____ colonies grew on plates seeded with a total of _____ cells

EMS induced $lac^+ \rightarrow lac^-$

_____ confirmed white colonies among _____ colonies screened

EMS induced $rif^S \rightarrow rif^R$

_____ colonies grew on plates seeded with a total of _____ cells

Spontaneous reverse mutation: $lac^- \rightarrow lac^+$

Class data will be posted: do not pool data for your report – rate for each lac- strain should be considered individually

For each lac- strain examined:

Name _____ : _____ colonies/ _____ cells plated

Name _____ : _____ colonies/ _____ cells plated

etc.

Results: Tables summarizing the results of your *class data* workup:

1. mutation frequencies (see box below)
2. ratios of mutation frequencies

1. *Express mutation frequency (mutant cells/total) in two ways:*

Example

2.5×10^{-4} [$lac^+ \rightarrow lac^-$] mutant cells per total cells scored

= one mutant cell per 4000 cells scored

Please be conservative about significant figures:

http://en.wikipedia.org/wiki/Significant_figures#Superfluous_precision

2. *Compare mutation frequencies by indicating ratios:*

- spontaneous lac^- /spontaneous rif^R
- induced lac^- /induced rif^R
- induced/spontaneous lac^-
- induced/spontaneous rif^R
- spontaneous forward/reverse mutations in lac utilization

Your **Discussion** of the data should address the following questions. Your discussion should be *in paragraph form* and will be graded according to its scientific content and overall coherence and organization.

1. How do the spontaneous and induced mutation frequencies compare for each gene? Be QUANTITATIVE. Don't tell me just that the induced mutation rate is higher than the spontaneous. Say the forward mutation rate is ?? times higher (ratio).
2. A mutant phenotype can result from a forward mutation that causes a loss-of-function or a gain-of-function. How would you classify the mutant phenotypes that we examined in this lab? For each gene, consider how a loss or gain-of-function would affect the organism. Include a discussion of the mode of action of rifamycin and also discuss what genes could be mutated to produce a lac^- phenotype. The variable size of the rif^R colonies suggests that some resistant cells grew faster than other. What could explain the difference in growth rates between different rif^R clones?
3. What are the various factors that determine mutation frequency and appearance of a specific mutant phenotype? Do you expect the mutation frequencies (either spontaneous or induced) to be the same for every gene?
4. How does the frequency of forward mutations compare to reverse mutations? Why is a reverse mutation different from a gain-of-function mutation?