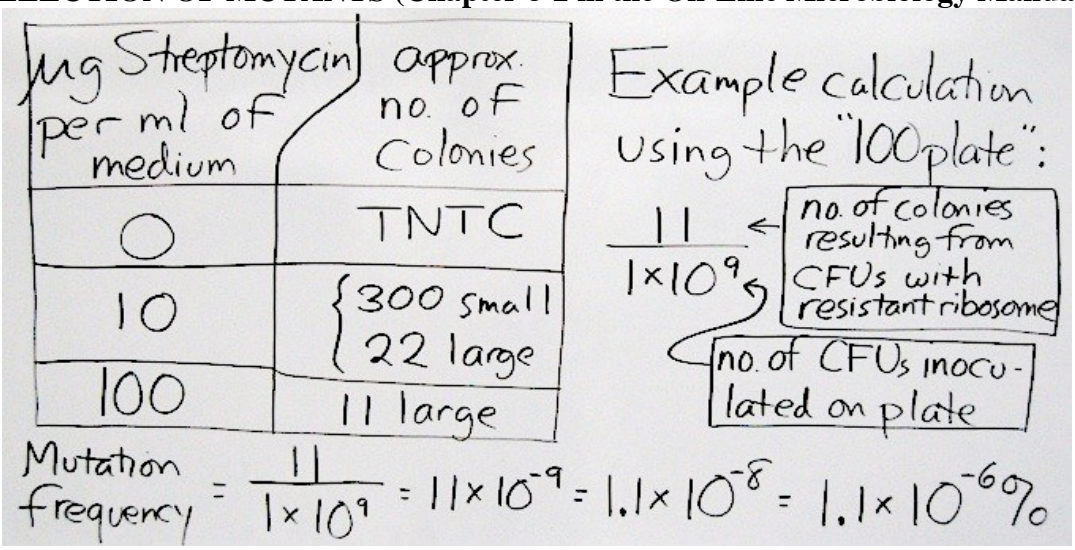
MICROBIOLOGY 102 – VIRTUAL EXPERIMENT 8B
SOLUTIONS TO ON-LINE VIRTUAL EXPERIMENTS CONCERNING MUTATION AND RECOMBINATION

Any colony counts derived from the images on the virtual experiments are very rough, and more may be found with a higher-resolution monitor than what was used to generate the solutions here. Whatever the colony counts, the basic methods for determining mutation frequency and recombination frequency are maintained. **To simplify matters for these problems, let's assume there is only one cell in a colony-forming unit** and we can use the terms interchangeably herein; this is only for the sake of simplifying these problems to learn what "frequency" is all about.

"Frequency" means the **number that did** *divided by* the **number that could**. So, for **mutation frequency**, it is the number of cells that did mutate *divided by* the number of cells in the total population, as any cell could conceiveably have been a mutant. For **recombination frequency** involving Hfr and F^- cells, it is the number of cells that did undergo detectable recombination *divided by* the number of cells that could have undergone recombination; in each case only F^- cells can receive DNA from Hfr cells and undergo recombination.

SELECTION OF MUTANTS (Chapter 8-2 in the On-Line Microbiology Manual)

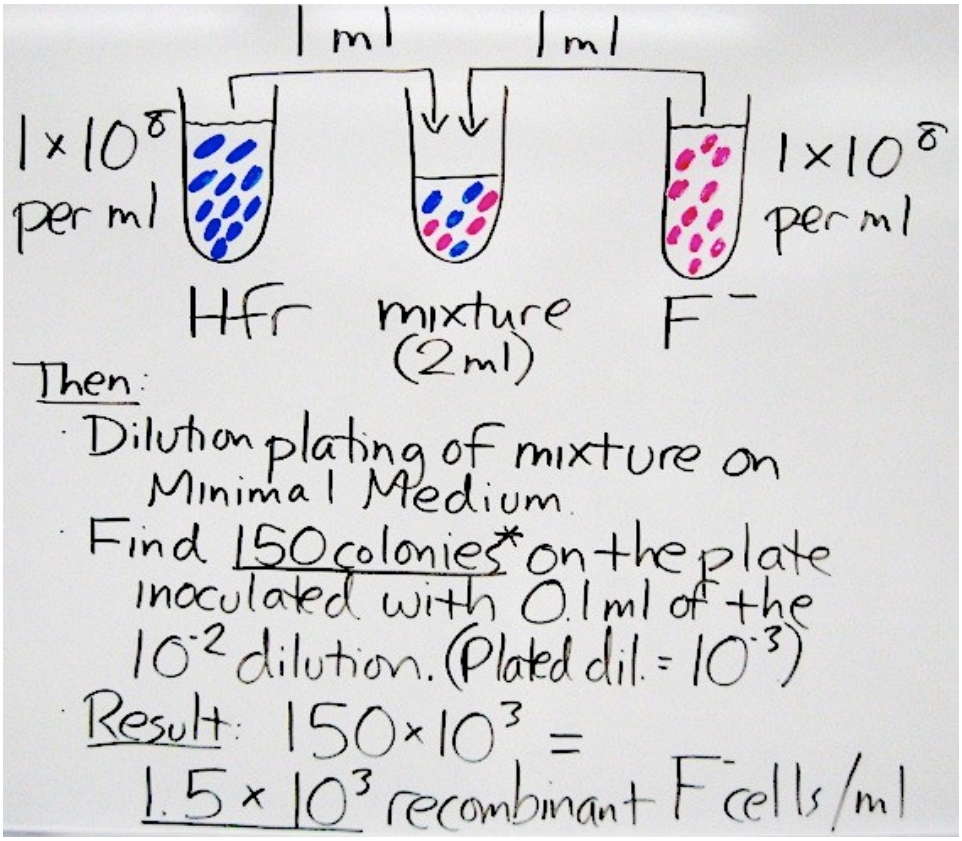


For our example calculation, we see 11 colonies on the plate of Nutrient Agar containing the high concentration of streptomycin (100μg of streptomycin per ml of the medium).

> The mutation allowed the colonies seen on the "100 plate" to not be affected by the high streptomycin concentration. The ribosome was altered by the mutation so it became resistant to the streptomycin whose actual "target site" is the ribosome. Thus, streptomycin has no effect and protein synthesis carries on.
>
> Those **smaller colonies** seen at the lower concentration of streptomycin have a mutation that affects the cell membrane such that nutrients and other things do not get into the cell through the membrane as easily as the normal situation. But once the streptomycin concentration is increased (as in the "100" plate), enough streptomycin can get into the cell to stop protein synthesis at the ribosome, and we see none of the smaller colonies at 100μg of streptomycin per ml of the medium.

The total number of cells inoculated onto the plate was **1 X 10^9** (from 0.1 ml of a cell suspension whose concentration was 1 X 10^{10}). Of those cells, only **11** were resistant to streptomycin and gave rise to the 11 colonies that we could count. So, the **mutation frequency would be 11 divided by 1 X 10^9**, and this fraction can be converted to a percentage as we see above in the diagram.

CONJUGATION AND RECOMBINATION
(Chapter 8-3 in the On-Line Microbiology Manual)



Let's throw out a number and say we find 150 colonies on the plate inoculated with 0.1 ml of the 10^{-2} dilution of the mixture. Therefore, that gives us a count of **1.5 X 10^5 recombinant cells per ml of the mixture**, as the dilution factor is 10^3 which is multiplied by the number of colonies on that plate. Those colonies came from F^- cells that were able to grow on the minimal medium – having received (by conjugation) and incorporated (by recombination) the necessary genetic information from the Hfr cells.

Therefore, we compare that number to the **total number of F^- cells per ml of the mixture.** How do we determine that number?

- When we add the one ml of each strain to obtain the mixture, we then have 2 ml containing 2 X 10^8 cells which is the same as 1 X 10^8 cells/ml. But now half of them are Hfr cells, and the other half are F^- cells.
- So, the number of pairs of cells (and therefore the **number of F^- cells**) is half of 1 X 10^8 which is **5 X 10^7 cells/ml**.

Based on the number of cells per ml of the mixture, the **recombination frequency** is the number who did undergo recombination (**1.5 X 10^5**) divided by the number who conceivably could have undergone recombination (**5 X 10^7**). This fraction can then be converted to a decimal or percentage.