CREATES - TE: Thinking About the Experiment

Questions / Things that were unclear:

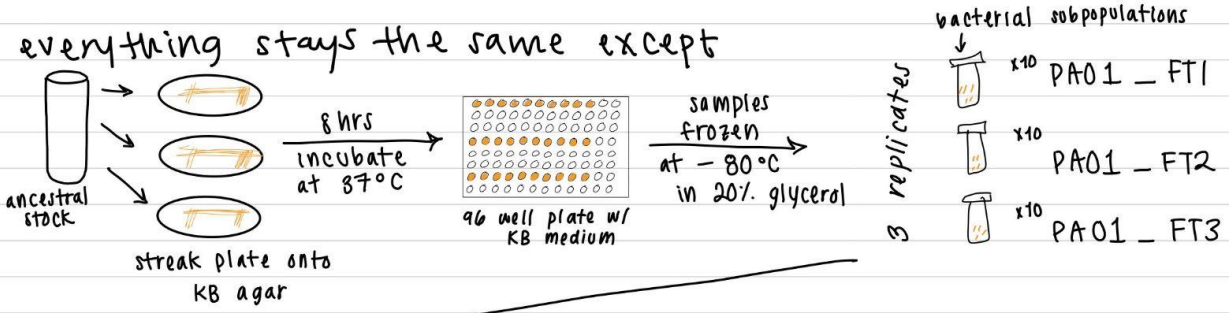
1. Why were the researchers unable to isolate PT7-resistant mutants? Was the phage response too strong?
2. Why were LPS and type IV pilus chosen as the representative receptor targets for this study? Did they only have access to phages that attacked those targets?
3. What exactly is the organization of the 96-well plate in the 2nd-step sequential selection phase of the methods? I cannot think of any more combinations other than the one I described. Was each combination repeated 3 times with each replicate? Is that what makes up the 12 columns in the 96-well plate?
4. How do these experiments translate to *in vivo* situations? Would replicating this experiment *in vivo* in mice be useful for building a real world model of the application of these experimental conclusions?
5. How could the dynamics of bacterial resistance change past 48 hours, since these samples were only incubated for less than 48 hours and bacterial infections oftentimes last longer than two days in real people.

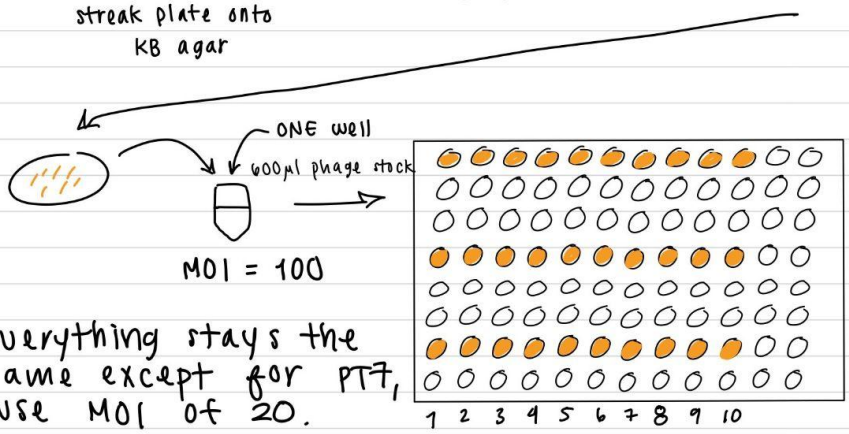
Brainstorming solutions:

1. Because the researchers hypothesized that the phage identity of PT7 was too strong and may have overwhelmed the effect of phage timing and order of exposure, I would like to see the experiment redone at a lower MOI. As opposed to using an MOI of 100, would there be a better chance of isolating PT7-resistant mutants if, for example, an MOI of 20 was used instead?
2. Although LPS receptors and type IV pili are common adsorption receptors for phages, it would be interesting to see what different outcomes arise when different targets are used. For example, what about phages that rely on host cell DNA polymerase to transcribe their genes? Could this DNAP be a target? Could there be a mutation in DNAP that confers resistance to phages? Would this be too fitness cost heavy?
3. To figure out this confusion, I will speak with my JC partner and instructor to get a better idea of this methods section.
4. Use mice infected with *Pseudomonas aeruginosa* PAO1 strain for at least 24 hours. Separate into 5 groups, one for each phage and one control (in the absence of phage). Infect each of the groups with the respective phages and monitor mice behavior and bacterial growth curve for 72 hours (is this possible in vivo?). Take blood samples every 24 hours to test for levels of *P. aeruginosa* in the blood.
5. Try different rounds of incubation. Have one group that is only incubated for 24 hours, another for 48 hours, another for 72 hours, and one for 96 hours. Isolate and sequence each of the phage-resistance mutants for mutations and observe if there are any changes in number and type of mutations for each group.

Brainstorming Idea #1

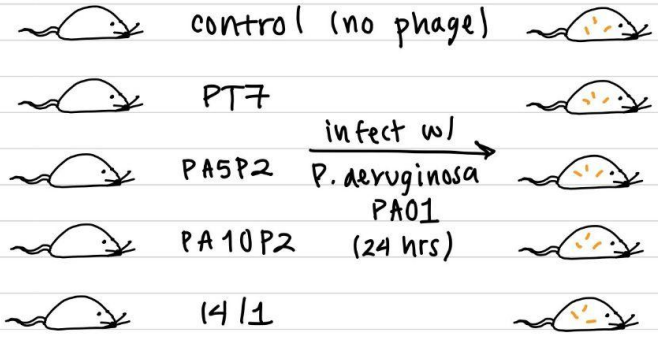
everything stays the same except





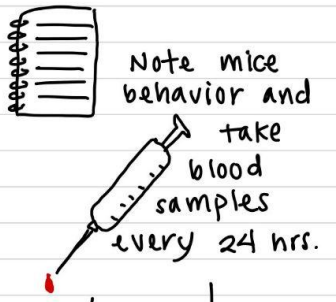
everything stays the same except for PT7, use MOI of 20.
20 phages per bacteria

Brainstorming idea #4



infect w/ P. aeruginosa PA01 (24 hrs)

introduce phages (monitor for 72 hrs)



test for levels of P. aeruginosa

sequence each blood sample for alterations in mutations over time.