E - Elucidate the Hypothesis

- Overall Research Question: How does the evolution, genetic basis (mutations), and cost of resistance against different phages targeting the same or different bacterial surface receptors change depending on the exposure to phages simultaneously or in succession (and the order of phages applied in succession)?
- Experiments and Hypotheses
    - Experiment 1: simultaneous exposure of phages - resistance
        - Type: experimental test
        - Hypothesis: Since the use of multiple phages together is thought to combat the development of resistance to phages, if multiple phages are effective in limiting development of resistance in bacteria, then simultaneous addition of phages to strain PAO1 will result in mutant bacteria with limited resistance to either phage applied.

- Experiment 2: sequential exposure of phages - resistance
    - Type: experimental test
    - Hypothesis: Since previous studies have shown that sequential exposure of phages can be as effective at limiting resistance as simultaneous application of phages, if sequential application of phages to a bacterial population is effective in limiting development of phage-resistance, then sequential addition of phages to strain PAO1 will result in mutant bacteria with limited resistance to either phage applied.
- Experiment 3: exposure of phages targeting the same cell surface molecule
    - Type: experimental test
    - Hypothesis: Since cross-resistance may occur more easily if multiple phages selecting for resistance target the same cell surface molecule, if cross-resistance occurs more often when bacteria are exposed to multiple phages targeting the same cell surface molecule, then when strain PAO1 is exposed to multiple phages targeting the same molecule, cross-resistance mutations will occur.
- Experiment 4: exposure of phages targeting different cell surface molecules
    - Type: experimental test
    - Hypothesis: Since cross-resistance may occur more easily if multiple phages selecting for resistance target the same cell surface molecule, if cross-resistance is deterred when bacteria are exposed to multiple phages targeting different cell surface molecule, then when strain PAO1 is exposed to multiple phages targeting different molecules, cross-resistance mutations will not occur.
- Experiment 5: simultaneous exposure of phages targeting different cell surface molecules - fitness cost
    - Type: experimental test
    - Hypothesis: Since mutations of cell surface molecules are associated with high fitness cost and since resistance to multiple phages likely requires multiple mutations, if resistance to multiple phages is associated with high fitness cost, then bacteria with resistance to multiple phages will have highly reduced fitness compared to bacteria with no resistance.
- Experiment 6: what / how many mutations accumulated in response to exposure to multiple phages

        - Type: descriptive test
        - Hypothesis: Since mutations are required for bacteria to gain resistance to phages, if a bacteria that is resistant to multiple phages exists, then bacteria resistant to multiple phages will show multiple mutations which mutate the targets of the phages the bacteria is resistant to.
    - Experiment 7: what / how many mutations accumulated in response to exposure of a bacteria to a single phage
        - Type: descriptive test
        - Hypothesis: Since mutations are required for bacteria to gain resistance to phages, if a single mutation is responsible for a bacteria that is resistant to a single phage, then the bacteria resistant to single phages will show limited (even single) mutations which provide resistance to the phage the bacteria is resistant to.
    - Experiment 8: what / how many mutations accumulated in response to simultaneous exposure of phages
        - Type: descriptive test
        - Hypothesis: Since cross-resistance mutations provide resistance against multiple phages more quickly, with a single mutation event, if cross-resistance mutations are more common in bacteria exposed to multiple phages simultaneously, then bacteria with resistance to phages applied simultaneously will have single cross-resistance mutations.
    - Experiment 9: what / how many mutations accumulated in response to sequential exposure of phages
        - Type: descriptive test
        - Hypothesis: Since sequential exposure of phages provides more opportunity for bacteria to accumulate multiple, specific mutations, if bacteria exposed to phages sequentially gain resistance to those phages through multiple mutations, then those bacteria will have multiple mutations, each specific for a certain phage target.
    - Experiment 10: how do specific mutations affect the fitness of the bacteria?
        - Type: descriptive test
        - Hypothesis: Since different mutations impose different levels of strain on a bacteria's fitness, if multiple resistance mutations have an additive or synergistic negative effect on bacterial fitness, then bacteria with multiple resistance mutations will have a higher fitness cost than bacteria with single resistance mutations.