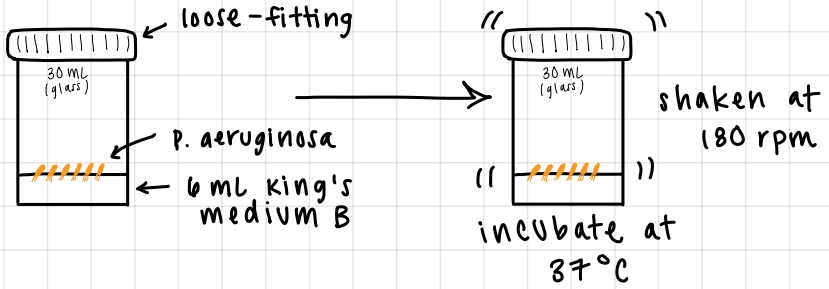
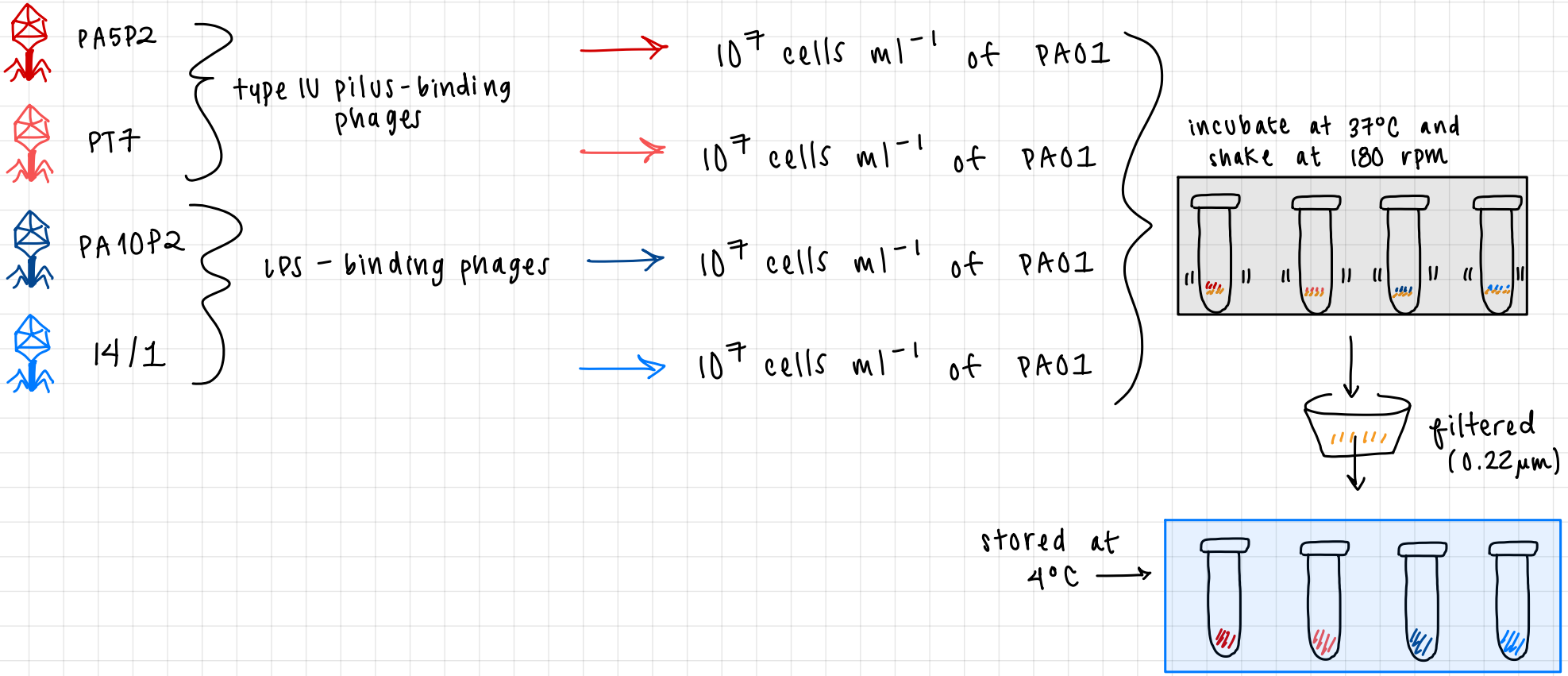
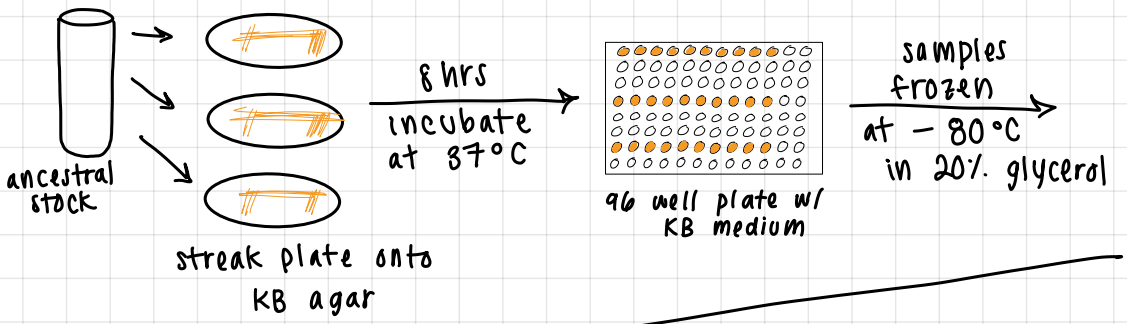
Materials & Methods

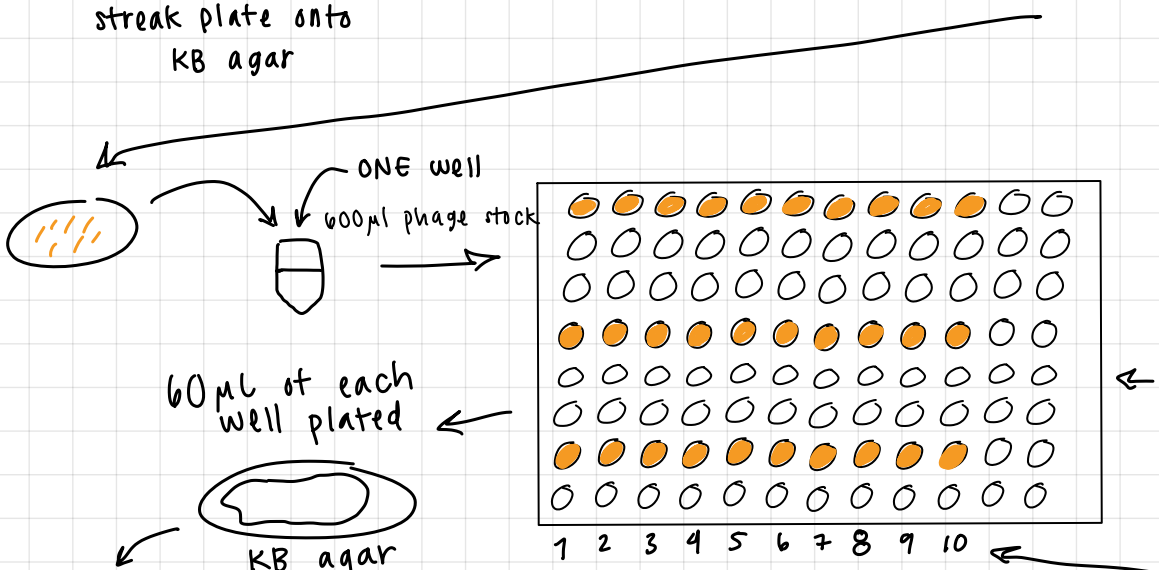
- strains and culture conditions:





selection of spontaneous phage-resistant mutants

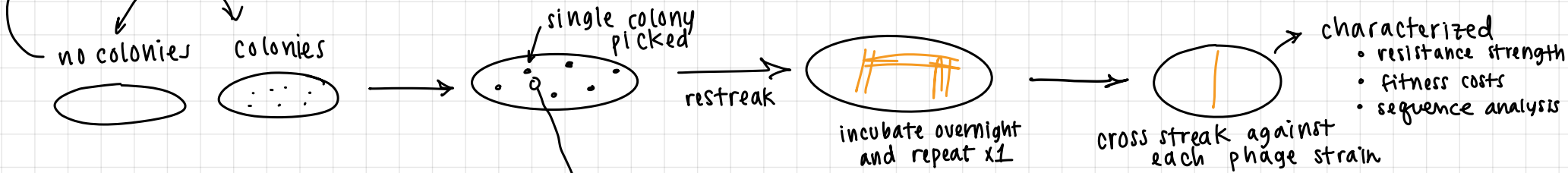




possible pairwise combinations:

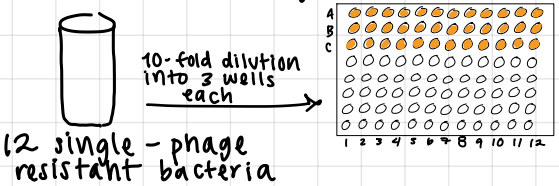
6 total

1	PA5P2 x PT7	LPS only
2	PA10P2 x 14/1	type IV pilus only
3	PA5P2 x PA10P2	LPS + type IV pilus
4	PA5P2 x 14/1	
5	PT7 x PA10P2	
6	PT7 x 14/1	



identified 12 resistant mutants selected against single phage strains
& 18 resistant mutants selected against two phages simulataneously

2nd-step sequential selection:



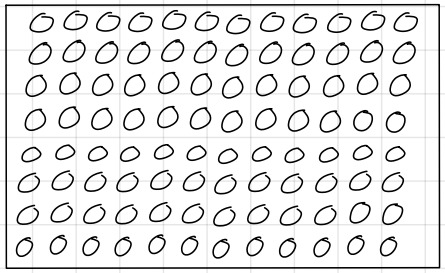
column 1: PA5P2 resistant bac + one of each phage bac. is not resistant to in A, B, C. x3
column 2: PT7 resistant bacteria + one of each phage bac. is not resistant to in A, B, C x3
column 3: PA10P2 resistant bacteria + " " x3
column 4: 14/1 resistant bacteria + " " x3

repeat same process above after second 96-well plate step

identified 36 phage resistant mutants

Quantitative resistance assays

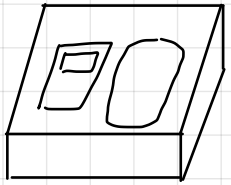
ij (w/ phage)



Includes all phage resistant bacteria + phages

MOI = 10 phage / bacteria

read absorbance at Abs = 600 nm at t=0 and t= 8 hours *



* for mutants with extremely slow growth, t=48 hours was used as the endpoint instead of t=8

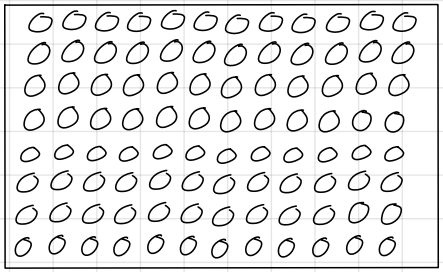
Equation used:

$$RBG_{ij} = \frac{[Abs_{600}(t=8h) - Abs_{600}(t=0h)]_{ij}}{[Abs_{600}(t=8h) - Abs_{600}(t=0h)]_{control}}$$

=

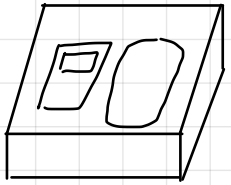
strength of resistance

control

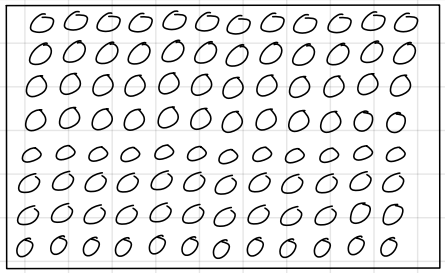


phage - resistant bacteria in the absence of phages

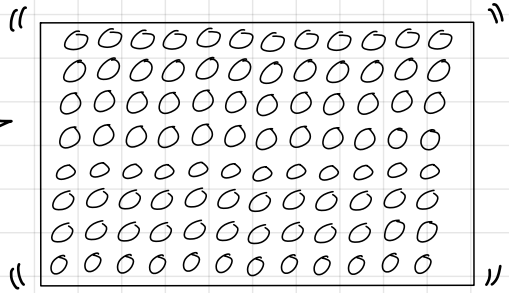
read absorbance at Abs = 600 nm at t=0 and t= 8 hours*



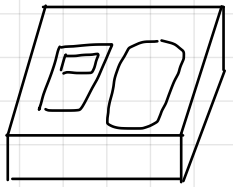
Relative fitness of phage-resistant mutants



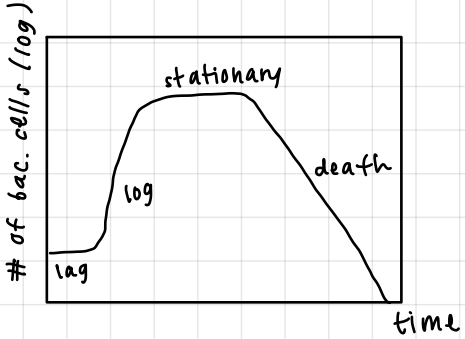
phage resistant bacteria in 150μl KB



incubate at 37°C and shake every 30 min when absorbance is taken



Take absorbance values for each well every 30 min at 600nm for 48 hrs.



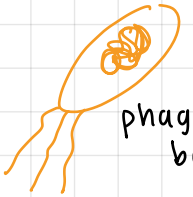
plot abs. values to create bacterial growth curve

characterize changes in bacterial growth:
- lag time
- max growth rate
- integral of growth curve
- max optical density

Calculate relative fitness:

$$\frac{\text{(max growth rate)}}{\text{(mean ancestral growth rate)}}$$

Sequence analysis of phage resistant bacteria



phage-resistant bacteria

→

A	T	G	C	A	T	T	C	G	
T	A	C	G	T	A	A	G	C	

Illumina MiSeq (sequence)

→ Detect resistance mutations

A	C	T	T	A	A	–	–	A	T	← resistance-mutated
T	G	A	A	T	T	G	C	T	A	← PA01 reference

Burrow-Wheeler Aligner

→ GATK Haplotype Caller used to identify SNPs and small indel variants

↓

ANOVA test to test for statistical significance

Statistical analysis

Multiple ANOVAs to analyse the effect of selection regime (simultaneous vs sequential exposure) on resistance and fitness

→ followed by post-hoc Tukey test
