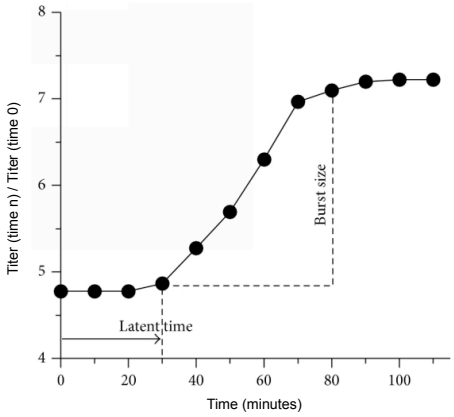
One-step phage growth assay (Stukey lab) – protocol v170526

The objective of this experiment is to determine the latent period and burst size associated with lytic growth of a phage at a particular temperature.

Basic experimental approach is to initiate a phage infection of cells and run a "synchronized" culture, assaying (via plaque assays) for phage growth over a time period of 240 minutes (some assays may need more time), collecting samples every 15 minutes starting at time 15-min. The experiment is done at a defined test temperature (e.g., 30°C, 32°C, 37°C, 42°C).

A short-time test run(s) to check that a proper number of plaques is achieved (for each specific phage stock and dilution-volume used) during early time points is advised.

Model one-step growth curve data:



Materials:
M. smegmatis culture – log-phase growth
Phage stock
Prep 40mL 7H9/AD/1mM $CaCl_2$, pre-warmed to test temperature. Will need 3 additional sterile 50-mL conical tubes for the following aliquots: 1 with 9.9-mL, 2 with 9.0-mL)
≈ 60 base agar plates for plaque assays
≈ 60 test tubes with 0.5mL *M. smegmatis* P2FF cells for plaque assays

Note: pre-warmed refers to test temperature.

Protocol:
Day-1
1. Inoculate 20-mL 7H9 culture from P1FF stock with volume to yield culture at OD_{600} ≈ 0.200-0.250 next (assay) day. Multiple cultures (2 or 3) inoculated with different volumes can help assure you have a culture ready to go next morning.
2. Incubate at test temperature with shaking (e.g., 37°C, 250 rpm).

Assay Day
1. Collect log-phase *M. smegmatis* cell culture at OD_{600} ≈ 0.200-0.250 (cells concentration ≈ 3-4 x 10^6 cfu/mL)

2. Centrifuge 12-mL volume of cells (use 15 mL conical tubes and table top centrifuge in lab at a setting of 6-7 for 5-10 minutes, or the Jouan centrifuge at 3k rpm for 10 minutes)
3. Re-suspend cells in 1.2 mL pre-warmed 7H9/AD/$CaCl_2$ medium (cells at ≈ 3-4 x 10^7 cfu/mL)
4. Transfer 0.9 mL of concentrated cells into a microcentrifuge tube and place at test temperature.
5. Add 0.1 mL of phage, diluted to ≈ 1 x 10^7 pfu/mL to microcentrifuge tube with cells (titer of phage after transfer to cells ≈ 1 x 10^6 pfu/mL; m.o.i. ≈ 0.1-0.03) and hold at test temperature for 3 minutes. Need to complete through step 12 within 15 minutes.
6. Centrifuge microcentrifuge tube for 1 minute at 12k rpm.
7. Remove all of supernatant that contains free phage and discard.
8. Re-suspend cell pellets in 1 mL of pre-warmed 7H9/AD/$CaCl_2$ medium and mix well by tapping/inverting.
9. Transfer 0.1 mL into 9.9 mL of pre-warmed 7H9/AD/$CaCl_2$ medium in a sterile 50-mL conical tube and mix gently (10^{-2} dilution).
10. Transfer 1.0 mL from 10^{-2} dilution tube (step 9) into another sterile 50-mL conical tube with 9.0 mL of pre-warmed 7H9/AD/$CaCl_2$ medium and mix gently (10^{-3} dilution).
11. Transfer 1.0 mL from 10^{-3} dilution tube (step 10) into another sterile 50-mL conical tube with 9.0 mL of pre-warmed 7H9/AD/$CaCl_2$ medium and mix gently (10^{-4} dilution).
12. Hold all three tubes at test temperature.
13. Starting at Time = 15 minutes (post phage infection initiation, step 5), gently mix and collect 30 µL and 90 µL volumes from each dilution tube (or relevant dilution tubes) and use in plaque assays (Time n), immediately adding TA and plating samples.
14. Repeat every 15 minutes through end time point (time points 0 – 240 minutes in 15-minutes increments). Timing intervals can vary a little at the start of assay in which collections may be extended up to 30-minute increments but it is best to keep intervals no longer than 15 minutes during the middle of the assay to better capture data points for determining latent period and burst size.
15. Incubate plates at 37°C for up to 24 hours and then count plaque numbers per plate on those showing between 20-300 plaques (depending on plaque size). Use all useful plate counts to determine the titer at that time point.
16. Use 15 and 30-minute times points (at minimum, but could be longer up to detection of new phage release) to calculate the ≈ Time = 0 titer. All other time point titer calculations will be divided by this ≈ Time = 0 value.
17. Plot: titer n/titer 0 over time.

Data evaluation:

1. Average early time point titers (15-30/60 min) to determine Time = 0 value.
2. Examine data in sequence for increase in titer ratio. Identify the time range from that showing the last "0" time value through at least 2 increases (sometimes this may be 3 increases; ideally like to see double digit ratio increase). Use the midpoint time for latent period measurement.
3. Examine data for 'plateau' in titer ratio and average all plateau data values for burst size measurement.