

UNIVERSITY OF OREGON  
INSTITUTE OF MOLECULAR BIOLOGY  
EUGENE, OREGON

February 9, 1965

Dr. Arthur Kornberg  
Dept. of Biochemistry  
Stanford University School of Medicine  
Stanford Medical Center  
Palo Alto, California 94304

Dear Arthur:

We are sending you under separate cover a lysozyme deletion mutant of T4B: T4B e G19. This deletion (isolated by J. Emrich) covers all of the (mapped) lysozyme region.

Joyce Emrich in my lab has isolated a temperature sensitive mutant that <sup>(is apparently defective with respect to</sup> ~~apparently interferes with~~ the phages' ability to repair the cell wall after infection. At 43° cells infected with lysozyme-less mutants that in addition carry this other mutation lyse (though poorly) at the normal time of lysis even though no lysozyme is present. Cells infected with lysozyme-less mutants of course do not lyse at the normal lysis time. Experiments concerning all this are still in progress.

To make stocks of the lysozyme deletion-mutant:

1) Plate e G19 on Citrate+EWL (see below) and after about 6 hours incubation at 37° pick a single plaque with a small-diameter glass tube and add the plaque to about 1 ml buffer; store in cold for at least two hours.

2) Plate about 10<sup>4</sup> phage on citrate+EWL plates and incubate at 37° for about 6 hours. The number of plaques on the plate should be such that the plaques are just confluent. (We usually plate 0.1ml, 0.2ml and 0.4ml amounts from the buffer containing the picked plaque on each of three plates and choose the best plate).

3) Expose the plate to chloroform vapors (by adding about 1ml of chloroform to the top of the glass petri plate and leaving the inverted plate at room temperature for about 15 minutes). This prevents adsorption of phage to bacteria or bacterial debris.

4) Scrape soft agar layer into centrifuge tube containing 4.5ml tryptone broth (10 gm tryptone, 5 gm NaCl per liter water) and buffer (pH 7.0, 0.1M phosphate +  $10^{-3}$ M  $Mg^{++}$ ) (1/2 and 1/2 is easiest). Pipette up and down several times with a 10 ml pipette to break up agar and leave at room temperature for at least 15 minutes.

5) Centrifuge for 15 minutes at low speed (4,000 to 6,000 rpm in tabletop centrifuge) to sediment agar and debris.

The titer of such a stock should be about  $5 \times 10^{10}$ . If stock is to be further purified filter through celite first and then proceed with purification.

Larger volumes may be made from a plate stock by infecting log phase bacteria (in tryptone broth) at about  $2 \times 10^8$  bacteria per ml, with a multiplicity of 2 to 5 phage per bacterium, aerating for about 30 minutes, and then adding chloroform, shaking once or twice, and adding about 20  $\mu$ g EWL per ml and incubating at  $37^\circ$  for ten or twenty minutes till bacteria have lysed. Treat with DNase and centrifuge at low speed to remove debris. The titer will be about  $2 \times 10^{10}$ . For somewhat higher titers infect as above, add a second input of phage about 13 minutes after the first one, and aerate for about 60 minutes, then chloroform, EWL etc.

To assay plate on citrate+EWL plates.

Some scattered thoughts concerning the fragility of phage-infected cells:  $10^{-3}$  to  $10^{-2}$  M  $Mg^{++}$  often helps prevent the leaking of materials from infected bacteria; 0.1mg/ml gelatin may help protect infected bacteria against surface denaturation; resuspending infected bacteria by pipetting up and down very often annoys them and we usually resuspend by agitation; infecting with a multiplicity of three or four often yields sturdier bacteria than infecting with a higher multiplicity; T4-infected bacteria are often sturdier than T2H-infected bacteria; T2L-infected bacteria are sturdier than T2H-infected bacteria.

Citrate bottom agar:    10 gm tryptone  
                          11 gm Difco agar  
                          5 gm NaCl  
                          1 liter water

autoclave and cool to  $45^\circ$ ; just before pouring  
add:

50 ml Tris; pH 8.0; 1M  
10 ml 25% Sodium Citrate.  $2H_2O$   
pour 36 to 40 ml per plate.

Dr. Arthur Kornberg

- 3 -

February 9, 1965

Citrate top agar: same as bottom except:

7 gm "Noble" agar (ordinary agar sometimes  
doesn't work)

The top agar (only) is supplemented with 500 µg Egg white lysozyme (EWL) per 2.5 ml agar just before plating. We use 0.5 ml log phase B grown to  $2 \times 10^8$  per ml per 2.5 ml soft agar for plating.

I hope some of this may help!

With best regards,

Sincerely,



George Streisinger

GS:mm