

UNIVERSITY OF ILLINOIS
Department of
CHEMISTRY AND CHEMICAL ENGINEERING
URBANA

The William Albert Noyes Laboratory

Aug. 13, 1956

Dear Josh,

I have sent you separately cultures of Sh and Sh/s.

Sh = *Shigella dysenteriae*; Sh/s = Sh made resistant to streptomycin by "training". Both of these are from Joe Bertani who received the original Sh from you.

The Plk sent previously was freed of λ by several passages on Sh.

Here are the recipes for preparing lysates:

- 1) Confluent lysis plates (see my transduction paper on K-12 strains for media)

To a melted soft agar tube add 10^6 Plk and 1-2 drops of an overnight culture of the donor cells. Overlay on a thick (≈ 4 sec.) plate of agar + Ca^{2+} ($2.5 \times 10^{-3} M$).

As soon as layer hardens (≈ 5 min) put in $37^\circ C$ incubator.

Plaques should be confluent at 5-7 hrs. At this time add ~4cc broth to each plate. incubate for 2-5 hrs. further. Decant liquid, chloroform heavily, spin off debris. Keep upate in icebox with chloroform. Before using, acetate chloroform away or remove by dilution.

For some strains of K-12 it may be necessary to use as much as 10^7 plaque per plate. The tricks here are in knowing when the plates are ready for the broth and in knowing when you are using too much or too little phage.

Have confidence in your ability to raise this problem.

2) liquid upates

To a young culture at $5-10 \times 10^7$ l.u. add a multiplicity of 5-10 Plkc and enough Ca^{++} to bring to $2.5 \times 10^{-3} M$. Culture may be done in 3-8 hrs (or maybe ∞). This method may or may not work. Our results this way are unreliable, but it is very easy and it works.

I will not be in CSH. We are leaving on a
camping trip to Colorado and New Mexico in a few days.
We return on Sept. 10.

If there is more information you need, please
write me again. Hope you have good results
with PI trans-actin.

Sincerely

Ed Umox