Start the overnights

Day 0

Make 2 ml LB 30°C overnights of the strains from which the P1 lysates are to be prepared. Do not add any antibiotics to the overnights.

Preparation of a $P1_{vir}$ Lysate

Day 1

1. Place 0.25 ml of an LB overnight that the $P1_{vir}$ lysate is to be made from into 20 ml of LB media that contains 10 mM $CaCl_2$, 0.2% w/v glucose (20 ml + 0.2 ml 1M $CaCl_2$ + 80 µl 50% glucose).
2. Incubate the 20 ml culture in a shaking 37°C water bath until the OD_{600} is around 0.5. This will take 1½ to 2 hours. Do not use cells that have an OD_{600} of greater than 0.6, or the resulting lysate will be poor. If the cells overgrow dilute them in fresh LB media that contains 10 mM $CaCl_2$, 0.2% w/v glucose.
3. Place 20% excess of $P1_{vir}$ lysate stock into a microfuge tube and microfuge for 5 minutes at 5°C. Transfer all but 50 µl of the lysate into a new microfuge tube (avoid touching the bottom of the tube to stay away from pelleted cell debris).
4. Make a 10^{-1} dilution of a $P1_{vir}$ phage stock using ice cold LB that contains $CaCl_2$ added to 10 mM as a diluting medium. Keep both the $P1_{vir}$ phage stock and the $P1_{vir}$ phage dilution on ice until ready for use.
5. Place 0.5 ml of the cell culture into each of two sterile 16 × 150 mm culture tubes labeled concentrated and 10^{-1}, then add 100 µl of the appropriate $P1_{vir}$ phage stock to each of the tubes.
6. Vortex the tubes well then incubate them in a 37°C water bath for 25 minutes. While the cultures are incubating, microwave some L Top then add $CaCl_2$ at a final concentration of 5 mM to the dissolved L Top. (45 ml L Top + 225 µl 1M $CaCl_2$ + 180 µl 50% glucose, maintain around 55°C)
7. Add 4 ml of L Top that contains 5 mM $CaCl_2$ to each of the culture tubes, vortex at a medium setting, then pour the mixture on a TYE plate labeled with the strain from which the P1vir lysate is being made plus whether the $P1_{vir}$ phage stock or 10^{-1} dilution was used.
8. After the L Top has hardened place the plates inverted in a 37°C incubator overnight.
9. Make a 2 ml 37°C LB overnight of the recipient strain.

Harvesting the Plate Lysate

Day 2

Remove the plates from the 37°C incubator and examine them. To get the best $P1_{vir}$ lysate you want to use the plate that initially contained the least amount of $P1_{vir}$ yet still resulted in confluent lysis.

If there is confluent lysis on both of the plates then harvest the $P1_{vir}$ from the 10^{-1} plate. If there is not confluent lysis on the 10^{-1} plate and you can still see individual plaques then harvest the $P1_{vir}$ from the concentrated plate.

1. Remove the plates from the 37°C incubator and place them in the refrigerator for 1 hour or longer.
2. Prepare 5 ml of LB which contains 10 mM $CaCl_2$ for each plate and place it on ice.
3. Add 5 ml of the ice-cold LB which contains 10 mM $CaCl_2$ to each of the plates. Add 0.5 ml of $CHCl_3$ to this, replace the lid and put the plate back in the refrigerator for 1 hour or longer.
4. With a 5 ml pipette scrape off the top agar in order to mix the lysate.
5. Pour both the liquid and top agar into a Falcon tube, add 1 ml of $CHCl_3$, vortex well, cap it, then place the tube on ice for 10 minutes.
6. Centrifuge at 7000 rpm for 15 minutes.
7. With a Pasteur pipette transfer the supernatant into a clean centrifuge tube (stay away from the bottom, i.e., chloroform and cell debris).
8. Spin again at 7000 rpm for 15 minutes.
9. Decant the supernatant into a screw cap centrifuge tube, label it and store in the refrigerator.

Doing a $P1_{vir}$ Transduction

Day 1

1. Place 200 µl of an LB overnight of the strain that is to be transduced into 2 ml of LB broth that contains 5 mM $CaCl_2$ (10 µl 1M $CaCl_2$). Use a sterile 16 × 150 mm culture tube.
2. Let the culture roll in a 37°C incubator for two hours.
3. Obtain six sterile microfuge tubes, label them with the strain that is to be transduced and mark them cells only, phage only, concentrated, 10^{-1}, 10^{-2}, and 10^{-3}.
4. Make a 10^{-1}, 10^{-2}, and 10^{-3} dilution of the $P1_{vir}$ lysate using LB that contains 10 mm $CaCl_2$.
5. Place 100 µl of the culture that is to be transduced into five of the six tubes (do not put any cells in the "phage only" tube), and then add 100 µl of the appropriate dilution of the $P1_{vir}$ lysate to each of the tubes (do not add any phage to the "cells only" tube). Vortex all of the tubes at a medium setting.

   One of these amounts of Pl_{vir} will yield a good level of transductant colonies.

   The broad range in the concentrations of $P1_{vir}$ that are used for transductions reflects three points: (a) the titers of $P1_{vir}$ phage can vary greatly depending on the host that they are prepared from; (b) the titer of $P1_{vir}$ phage prepared from a plate lysate will be 10-100-fold higher than the titer of $P1_{vir}$ phage prepared from a liquid lysate; (c) if the same $P1_{vir}$ phage stock is used, one will obtain 10-fold more sugar or amino acid transductant colonies than antibiotic transductant colonies.

6. Place the tubes in a 37°C standing water bath for 25 minutes.
7. Add 0.2 ml of 0.1M Sodium Citrate to each of the tubes, vortex well, and microfuge for 5 minutes. Discard the supernatant, resuspend the cell pellet in 0.1 ml of 40 mM Sodium Citrate and plate the cells on the proper type of plate that has been appropriately labeled with the following information:
   a. The strain that is being transduced.
   b. What the strain is being transduced to; i.e., the marker that is being moved into the strain.
   c. Which dilution of $P1_{vir}$ was used to do the transduction.

The following nomenclature is commonly used to denote this information:
(Strain that P1 lysate was made from) / (dilution used) → (Recipient Strain)

For example, if a P1 lysate was made to move the zjb::Tn5-20 transposon insertion from strain EA1100 into the recipient strain MC4100 and a 10^{-2} dilution of the lysate was used, this information would be abbreviated as EA1100/10^{-2} → MC4100

8. Let the plates dry then place them inverted in a 37°C incubator.

Day 2 or Day 3

Single colony transductants will appear overnight if a rich plate containing antibiotics was used to screen the recombinants. If a minimal plate containing the appropriate sugar or lack of a specific amino acid was used to screen the recombinants then it will take two days to get single colony transductants.

1. Pick a single transductant colony and restreak it for single colonies on the same type of plate that was used to screen transductants.
2. When the plate is checked for single colonies in one to two days depending on the plate used, plaques will be apparent in the heavy part of the streak. These are $P1_{vir}$ that were transferred from the transductant plate. Pick a single colony from this plate for subsequent use.