Period 7 (Mar. 12)

Strain construction using phage P1-mediated transduction.

Background: When phage (bacterial virus) P1 infects *E. coli* and replicates inside it, pieces of *E. coli* DNA are occasionally encapsulated into the P1 protein shell. (WHAT IS NORMALLY ENCAPSULATED?). When these pieces of DNA are subsequently injected into a second strain of *E. coli*, they can recombine into its chromosome by homologous recombination. (WHAT IS HOMOLOGOUS RECOMBINATION?) To isolate a strain in which a recombinational event has occurred at a particular position on the genome, one must employ conditions under which recombinants will grow but the recipient strain of *E. coli* will not. The conditions employed are called selective conditions and the recombinants are called transductants. To understand transduction you must be able to draw diagrams of the processes that are occurring (e.g. see below). This will be reviewed in Discussion Section.

Because we will study the enzyme β-galactosidase, the gene which encodes it (*lacZ*), and regulation of expression of the lactose (*lac*) operon in later exercises, we will illustrate the use of P1 transduction by repairing a deletion of the *lac* operon.

Materials:

1. Sterile Eppendorf tubes (50/pair)
2. Eppendorf centrifuges
3. Pipettemen and tips of various sizes
4. Hockey sticks and ethanol
5. Bunsen burners
6. Water bath at 37°C and Eppendorf tube floaters
7. Phage P1 grown on our wild-type strain of *E. coli*, which is lac^+ trp^+ (0.5 ml/pair)
8. Fresh stationary phase cultures of two *E. coli* recipient strains that have been concentrated 5-fold in P1 broth (see attached) (2 ml/pair). Strain E1458 [= RK4353 (Δ*lac*)] carries a deletion of the *lac* operon (Δ*lac*) in a genetic background that is different from that of the *E. coli* K12 wild-type strain we usually employ. Likewise, strain E3912 (Δ*trp*) carries a deletion of the tryptophan biosynthetic operon (Δ*trp*) in a background that is different from that of our *E. coli* K12 wild-type strain. The strains have been concentrated as described in Methods below.
9. Sterile phage dilution buffer (see attached) (5 ml/pair).
10. Sterile LB (20 ml/pair)
11. Sterile sodium pyrophosphate (see attached) (12.5 mM; 5.5 g/l) (1 ml/pair)
12. N^-C^- plates containing lactose (0.4%) and NH_4Cl (10 mM) = "N^-C^-lactose" (10/pair)
13. Ice for P1 phage
14. One LB plate/pair
15. Stop watches
16. Sterile glass culture tubes to dilute phage
17. Test tubes and test tube racks for taking samples

Methods:

Transduction with P1*vir*. **You will need to write a protocol for this.**

A. Grow the recipient strain of *E. coli* to stationary phase in 5 ml P1 Broth (see attached). This will be done for you.

B. Centrifuge the cells for 5 min at 1500 g at room temperature. This will be done for you.

C. Suspend the cells in 1 ml P1 Broth. This will be done for you. Half the class will use E1458 (Δ*lac*) and half the class will use E3912 (Δ*trp*).

D. If E1458 is your recipient, add 0.1 ml of phage or phage diluted 10- or 100-fold in phage dilution buffer (cold) to a series of Eppendorf tubes. Be sure to include a tube with no phage as a control. Prepare an additional tube with concentrated phage to which you do not add recipient bacteria as a second control. WHAT IS THE PURPOSE OF THE CONTROLS?

   If E3912 is your recipient, add 0.1 ml of phage diluted 10-, 100-, or 1000-fold in phage dilution buffer (cold) to a series of Eppendorf tubes. Also prepare the control tubes described above.

E. Add 0.1 ml of concentrated recipient strain. Incubate for 20 min at 37°C in a water bath without agitation. This allows adsorption of phage and injection of DNA. THE TIME IS CRITICAL. IT SHOULD BE 20 MIN, NOT 15 OR 25. You need sufficient time for adsorption of phage and injection of DNA but not so much time that lysis begins.

F. Add 1 ml of LB containing 125 μM sodium pyrophosphate (which will chelate the Ca^{2+} and Mg^{2+} required for phage adsorption) PYROPHOSPHATE IS A PARTICULARLY EFFECTIVE CHELATOR AND GREATLY SIMPLIFIES TRANSDUCTION WITH PHAGE P1.

G. Centrifuge the cells for 30 sec at room temperature. You will need a sixth tube to balance the centrifuge. Speed 5 is adequate. Press the pulse button and time for about 30 sec. Decant the supernatant.

H. Suspend the cells in 1 ml of LB containing 125 μM pyrophosphate (LB + pyrophosphate).

I. Incubate 30 to 60 min at 37°C in a water bath without agitation. This allows additional time for the recombinational event to occur and for the new gene(s) on the chromosome to be expressed.

J. Centrifuge the cells for 30 sec at room temperature.

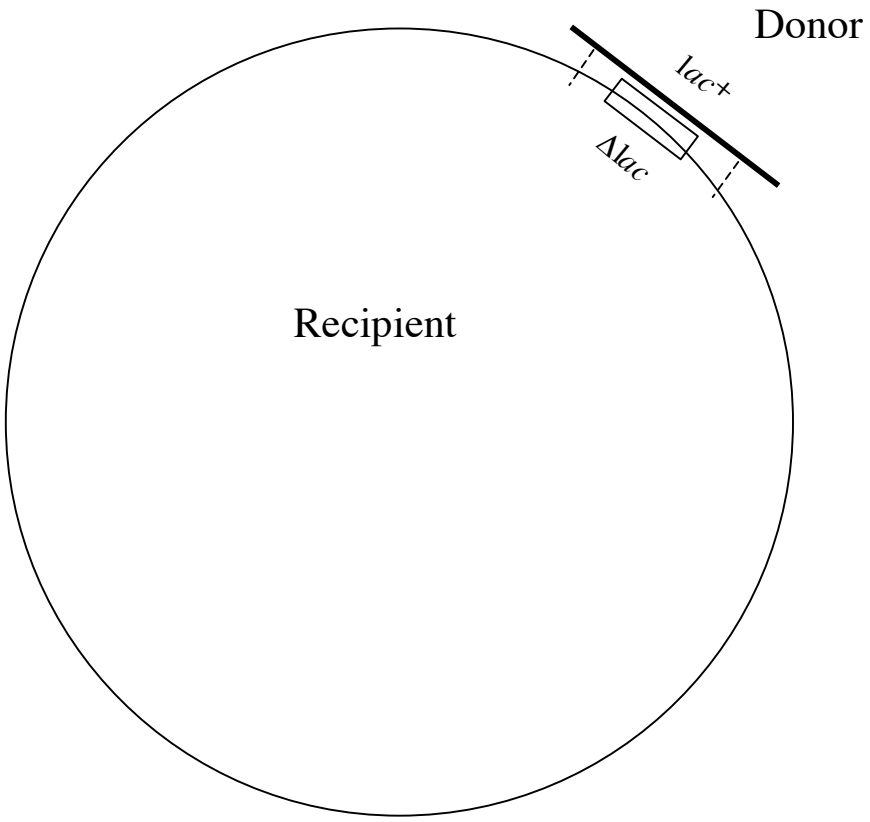
K. Suspend the cells in 0.1 ml LB + pyrophosphate and spread them on N^-C^- plates containing lactose as sole carbon source (0.4%) and NH_4Cl (10 mM) as nitrogen source (selective conditions). These plates do not contain pyrophosphate because its presence or absence at this point does not seem to matter. IS THIS A SATISFACTORY SELECTION FOR BOTH CROSSES?

L. Put one drop of undiluted phage on an LB plate.

M. Incubate the plates at 37°C for 48 hours.

Further Background:

Below is a diagram of the circular chromosome of *E. coli* (double-stranded, which is not indicated). The position of the *lac* deletion is indicated approximately. Outside the chromosome is a fragment of DNA (heavy line, not to scale), which is about 2.5% the length of the chromosome (100 genes). The diagram is designed to indicate that the fragment carries the *lac* operon. A fragment like the one diagrammed can be injected into a "recipient" strain from a P1 protein shell that carries *E. coli* DNA instead of P1 DNA. Such a fragment is picked up from a "donor" strain. Like the chromosome, the fragment is double-stranded, but unlike the chromosome, it is linear. Indicated by dashed lines are positions of representative double-stranded breaks and joints (recombinational events) that would yield a lac^+ transductant from the lac^- recipient. This will be reviewed in discussion section. P1 phage picks up all regions of the genome reasonably randomly when it mistakenly packages *E. coli* DNA rather than its own. For this reason it is called a generalized transducing phage. It can be used to transfer genes in any region of the *E. coli* chromosome.



Questions:

1. Describe selective conditions for isolating *lac*$^+$ transductants from a *lac*$^-$ recipient. (The strain on which P1 phage is grown is called the "donor" strain" for a transduction and the strain that is infected with it to isolate recombinants is called the "recipient" strain. In this case the donor strain is *lac*$^+$ and the recipient strain is *lac*$^-$).

2. Why will a *lac*$^-$ recipient strain not grow under the conditions you described in 1?

3. Suppose you want to construct a *lac*$^-$ strain by P1-mediated transduction. In this case your donor strain would be *lac*$^-$ and your recipient strain *lac*$^+$. Describe selective conditions for *lac*$^-$ transductants.

4. Will a *lac*$^+$ recipient strain grow under the conditions you have described in 3?

5. Describe selective conditions for isolating *trp*$^+$ transductants from a *trp*$^-$ recipient. Can you use the conditions described in 1?

6. Suppose you wanted to construct a *trp*$^-$ strain by P1-mediated transduction. In this case your donor strain would be *trp*$^-$ and your recipient strain *trp*$^+$. Describe selective conditions for the *trp*$^-$ transductants.

7. Will a *trp*$^+$ recipient strain grow under the conditions you have described in 6?

Solutions for P1*vir* transduction:

1. P1 Broth is LB containing 0.2% glucose and 5 mM $CaCl_2$.

2. A. Phage dilution buffer contains (per liter):
   10 mM Trizma base (0.6 g)
   10 mM $MgSO_4 \cdot 7H_2O$ (1.2 g)
   5 mM $CaCl_2 \cdot 2H_2O$ (0.4 g)
   50 mM NaCl (1.5 g)

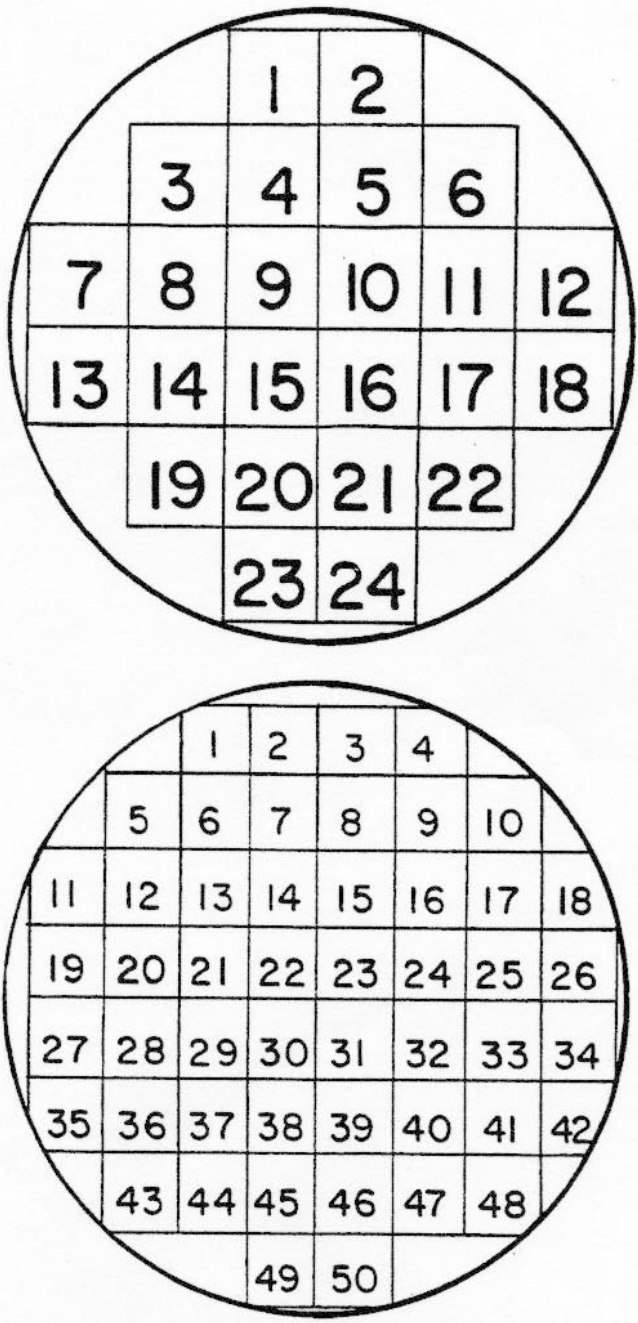
   B. Add water to 900 ml

   C. Adjust the pH to 7.5 with 0.1 M HCl and bring to 1 liter

3. The stock solution of sodium pyrophosphate ($Na_4P_2O_7.10\ H_2O$) is 12.5 mM. (HOW CONCENTRATED IS THIS STOCK SOLUTION RELATIVE TO THE FINAL CONCENTRATION YOU NEED?)

APPENDIX 11

PATCH PATTERN



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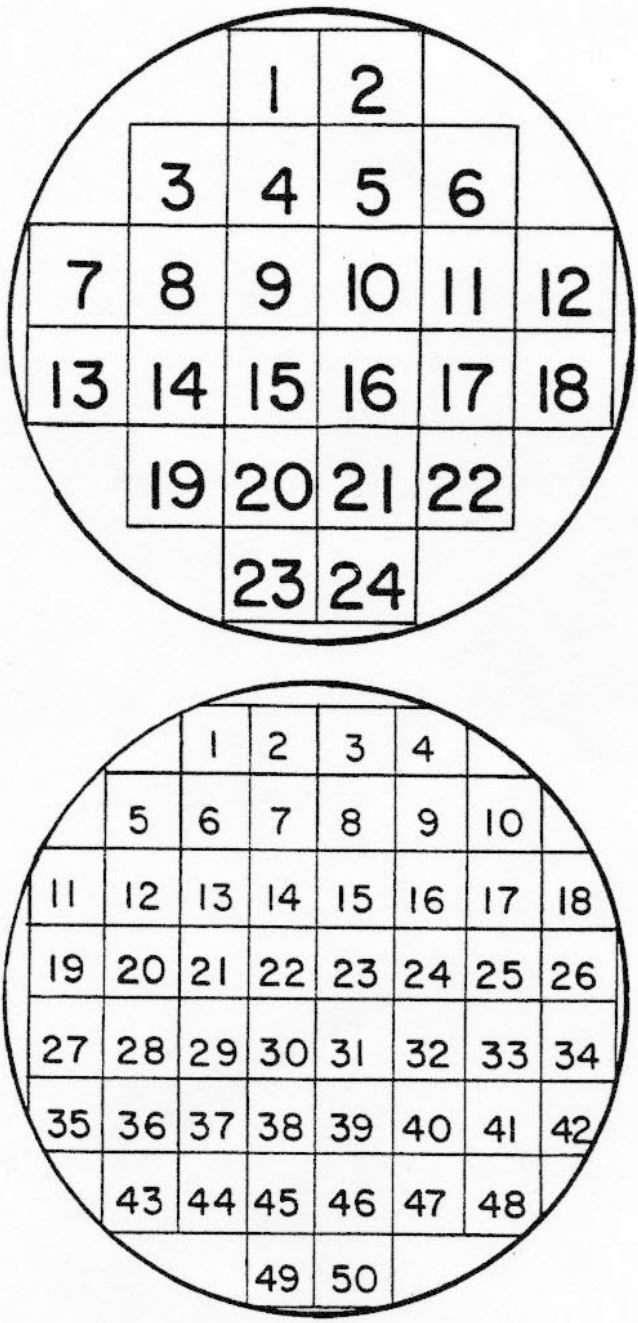

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