PHAGE P22

Growth of P22. P22 is a temperate phage that infects *Salmonella* by binding to the O-antigen, part of the lipopolysaccharide on the outer membrane. After infection, P22 circularizes by recombination between terminal redundancies at each end of the phage DNA. During lytic growth, the circular genome of P22 initially undergoes several rounds of θ-replication, then changes to rolling-circle replication. Rolling circle replication produces long concatemers of double stranded P22 DNA. These concatemers are packaged into phage heads by a "headful" mechanism: packaging is initiated at a specific sequence on the DNA called a *pac* site, then a nuclease moves down the concatemer, cutting every 48 Kb (Casjens and Hayden, 1988). Since the P22 genome is only 44 Kb, this yields the terminal redundancy at the ends of P22 (Susskind and Botstein, 1978). This linear double stranded DNA is packaged into new phage particles. When the cell lyses, it releases 50-100 new phage. (Diagrams of these events can be viewed on the Mcbio 316 page: http://www.life.uiuc.edu/micro/316/topics/phage/).

P22 lysates. After P22 infects a cell and bursts it, the released phage infect other cells in the culture. After many rounds of phage multiplication and lysis of a bacterial culture, the broth contains a high concentration of phage. Unlysed cells and cell debris are removed by centrifugation, and chloroform ($CHCl_3$) is added to kill any remaining cells, yielding a solution of phage called a phage lysate. P22 lysates can be stored for many years at 4°C with some $CHCl_3$ at the bottom of the tube to keep it sterile. (If cells grow in the lysate, the phage will adsorb to them, drastically decreasing the titer of the phage stock.)

Generalized transduction. There are sequences on the *Salmonella* chromosome that are homologous to the P22 *pac* site. When P22 infects a cell, occasionally the P22 nuclease cuts one of these chromosomal sites and packages 48 Kb fragments of chromosomal DNA into P22 phage heads. The P22 particles carrying chromosomal DNA (transducing particles) can inject this DNA into a new host. The DNA can then recombine into the chromosome by homologous recombination. Since P22 can transfer DNA fragments from all regions of the chromosome, this process is called generalized transduction (Masters, 1985; Margolin, 1987).

In this course we use P22 HT105/1 *int-201*, a P22 mutant that is very useful for generalized transduction. This phage has a high transducing (HT) frequency due to a nuclease with less specificity for the *pac* sequence. About 50% of the P22 HT phage heads carry random transducing fragments of chromosomal DNA (Schmeiger, 1972). The *int* mutation prevents formation of stable lysogens.

Unlike many phage, P22 is very "user friendly". P22 can infect overnight cultures, so you don't have to carefully monitor the growth of the culture to catch it in log phase. P22 nearly always produces high titer phage stocks with about 10^{10}-10^{11} plaque forming units (pfu) of phage per ml of lysate. Finally, P22 HT produces such a high percentage of transducing particles that it is easy to look for rare events, like recombination between very close genetic markers (Sanderson and Roth, 1983).

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USING PHAGE P22

A. Preparation of P22 phage lysates.

1. Pick a single colony and start an overnight culture of the donor strain in 1 ml LB at 30°C.
2. Add 1 ml P22 broth to 200 μl of the overnight culture. The final multiplicity of infection (moi) should be about 0.01-0.1 pfu/cell.
3. Incubate 8-16 hr in a 37°C shaker. (Temperature sensitive mutants can be grown at 30°C.)
4. Transfer the culture to a microfuge tube and centrifuge for 2 min at 12,000 rpm to pellet the cell debris.
5. Add several drops of $CHCl_3$and vortex. Store at 4°C. (A good lysate should contain 10^{10}-10^{11} pfu/ml.)

P22 Broth:

Mix the following to a 100 ml bottle of sterile LB broth:

2 ml	50x E medium (sterilized with $CHCl_3$)
1 ml	Sterile 20% glucose
0.1 ml	P22 HT *int* phage (sterilized with $CHCl_3$)

Store at 4°C.

B. P22 transduction.

1. Grow the recipient strain overnight in 1 ml LB.
2. Mix the cells and phage on selection plates as follows:

Plate	ml cells	ml P22	
A	200 μl	---	No phage control
B	---	5 μl	No cell control
C	200 μl	1 μl	
D	200 μl	5 μl	
E	200 μl	20 μl	

3. Dip a glass hockey-stick into alcohol then briefly pass it through a flame to burn off the residual alcohol. Thoroughly spread the plates with the alcohol-flamed glass spreader.
4. Place the plates in an incubator upside-down. Incubate overnight.
5. Count the colonies on each plate. There should be no growth on the cell or phage control plates. Any colonies you plan to save should be purified on EBU plates and cross-streaked against phage H5 to purify phage sensitive colonies (see section E below).

C. P22 transduction <u>with</u> phenotypic expression.

For certain transductions (for example, when selecting Kan^R or Str^R) phenotypic expression is required before plating on the selective medium. Phenotypic expression can be done in two ways:

- Spread the cells and phage on nonselective medium (e.g. LB plate), incubate 4-8 hrs, then replicate onto the selective plates. This replica plating approach usually gives more colonies and ensures that different colonies are not due to siblings.
- Mix cells and phage in a microfuge tube and incubate about 1 hr at 37°C before plating on the selective medium. Although this broth approach may yield somewhat fewer colonies and may produce some siblings, it is easier and faster than the replica plating approach.The broth method is detailed below.

1. Grow the recipient strain overnight in 1 ml LB.
2. Mix the cells and phage in sterile microfuge tubes as follows:

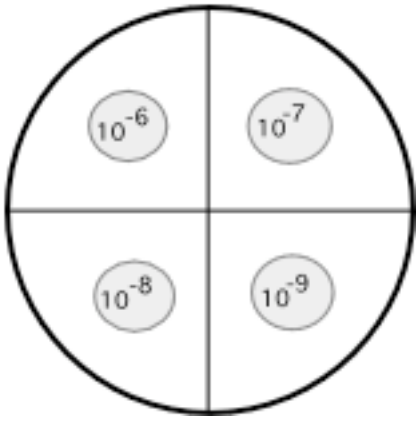
Tube	ml cells	ml P22	
A	200 μl	---	No phage control
B	---	5 μl	No cell control
C	200 μl	1 μl	
D	200 μl	5 μl	
E	200 μl	20 μl	

3. Leave at room temperature for about15 min to allow phage adsorption.
4. Add 1 ml LB and incubate about 1 hr at 37°C.
5. Centrifuge for 1 min in a microfuge.
6. Pour off the supernatent. Add 100 μl LB, and vortex to resuspend the pellet.
7. Dip a glass hockey-stick into alcohol then briefly pass it through a flame to burn off the residual alcohol. Thoroughly spread the plates with the alcohol-flamed glass spreader.
8. Place the plates in an incubator upside-down. Incubate overnight at 30-37°C.
9. Count the colonies on each plate. There should be no growth on the cell or phage control plates. Any colonies you plan to save should be purified on EBU plates and cross-streaked against phage P22-H5 to purify phage sensitive colonies (see section E below).

D. Spot titering P22 lysates.

When P22 HT *int* is used, phage stocks do not usually need to be titered. However, it is a good idea to check the titer of your phage stock if a transduction doesn't work.

1. Divide an EBU plate into 4 sectors with a marking pen. Make sure the surface of the plate is not noticeably wet. If the plate is wet, place it in an incubator or oven with the lid ajar until dry.
2. Melt TS top agar in a microwave. For each phage to be titered, add 2.5 ml of the melted top agar to a test tube and place in a 50°C heating block. After the top agar cools to about 50°C, add 0.1 ml of an overnight culture of wild-type *Salmonella* Pullorum to each tube.
3. Immediately swirl and pour onto the EBU plate.
4. Allow the top agar to solidify for 15-30 min.
5. Spot 20 μl of appropriate phage dilutions onto the lawn (usually 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} dilutions in sterile 0.85% NaCl).

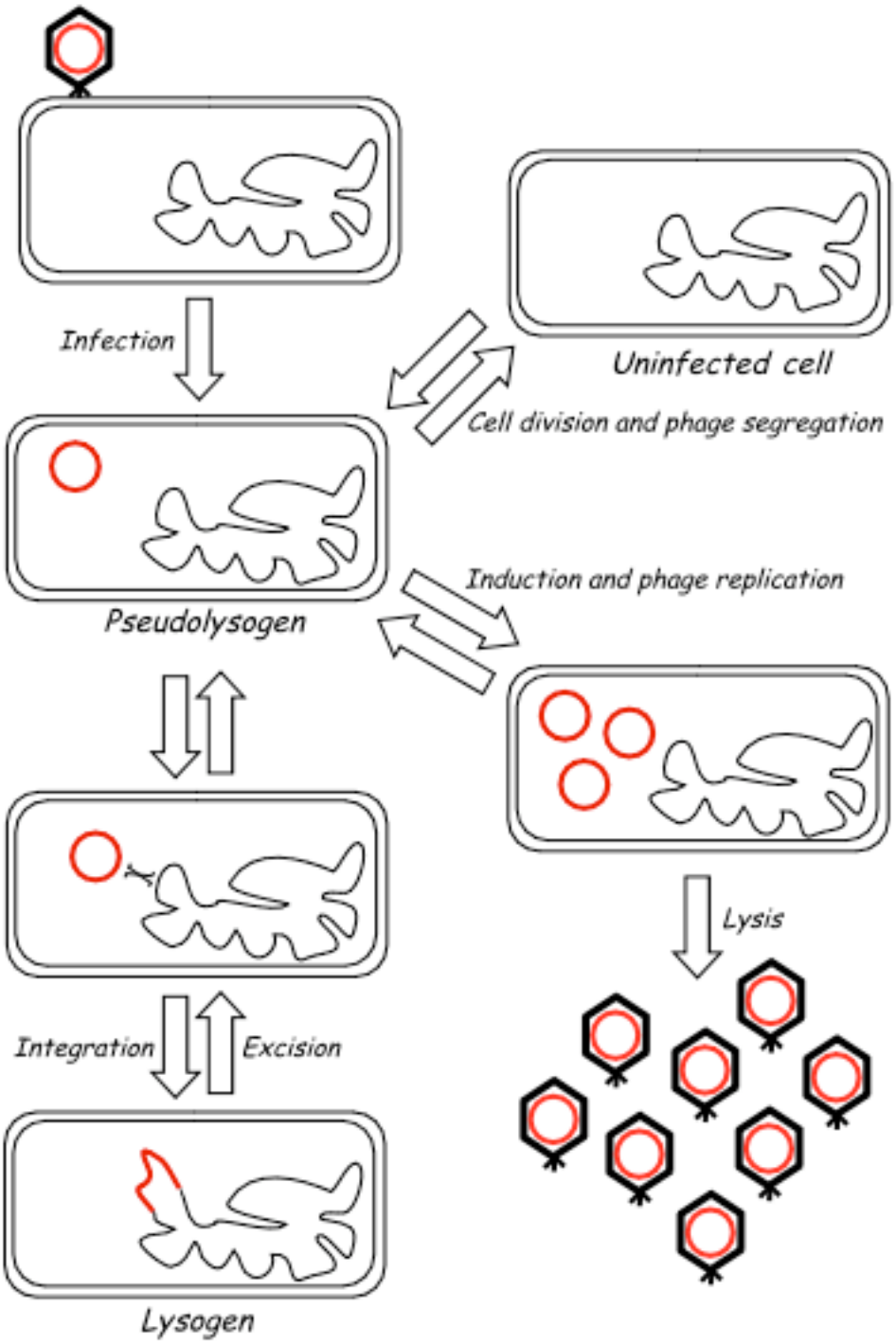


6. Leave the plate on the bench for about 30 min or until the drops of phage dry.
7. Incubate the plates upside-down at 30-37°C overnight.
8. Count the number of plaques in each spot. Each viable phage (plaque forming unit or pfu) will produce one plaque. Calculate the phage titer as follows:

$$pfu\ /\ ml\ =\ \frac{number\ \ of\ \ plaques \times\ \ dilution\ \ factor}{20\ \ \mu l} \times \frac{1000\ \ \mu l}{ml}$$

E. Checking *S. typhimurium* for P22 sensitivity.

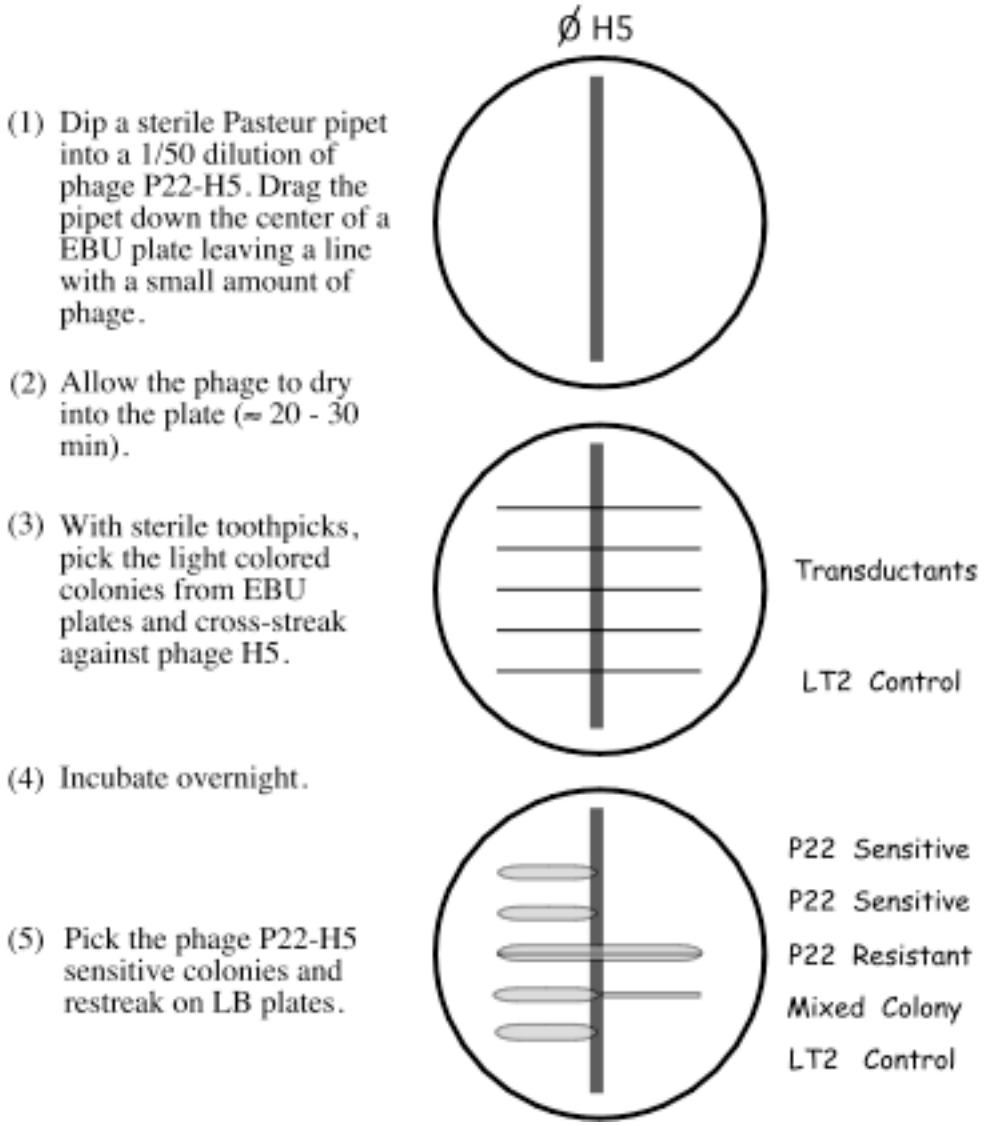
In addition to receiving a transducing fragment, some of the transductants may have also been infected with P22 phage. When *Salmonella* is infected with phage P22 the following pathways are possible (Bochner, 1984):



Pseudolysogens can be differentiated from nonlysogens and true lysogens on EBU plates. Green plates contain pH indicators which are green at neutral pH but turn dark blue at low pH. When streaked on green plates, nonlysogens and true lysogens form light-colored colonies. However, in a colony containing pseudolysogens many cells are undergoing lysis which lowers the pH of the medium resulting in dark blue colonies. Since in pseudolysogens the phage exists as a low copy number plasmid, it is possible to obtain "phage-free" segregants by streaking for isolated colonies on green plates. The "phage-free" segregants will form light-colored colonies while the pseudolysogens will remain blue.

P22 HT *int* is used for transductions. The *int* mutation prevents formation of stable lysogens. However, when cells are left on plates with lytic phage, there is a strong selection for revertants that form stable lysogens. Since stable lysogens cannot be reinfected with P22, such transductants are not very useful for genetic studies. Therefore, it is important to "clean up" transductants on green plates as soon as possible after a transduction.Ca^{++} is required for phage adsorption. EGTA chelates Ca^{++} so it is not available for phage adsorption. Therefore, we sometimes include EGTA in plates to decrease reinfection of transductants. (EGTA cannot be added to the initial selection plates or it will prevent adsorption of the transducing phage also). However, even colonies from EGTA plates must be cleaned up on EBU plates. Once light-colored colonies have been purified from EBU plates, they should be checked to make sure they are not true lysogens. This is done by cross-streaking against a P22 *c2* mutant called P22-H5. The P22 *c2* gene encodes a repressor equivalent to cI of phage lambda. Thus, P22 *c2* mutants produce clear plaques. Phage free cells are infected by H5 and lysed (H5 sensitive) but P22 lysogens are not infected by P22-H5 (H5 resistant). The test for phage P22-H5 sensitivity is done as shown on the next page.

Avoid "digging into" the agar when streaking EBU plates: cells ferment more glucose when anaerobic, so all of the colonies will appear dark blue. Also, the EBU phenotype should be observed promptly after growth appears: when left on EBU plates for many days, all of the colonies will turn dark colored.



Note that the efficiency of lysis may vary depending upon what concentration of P22-H5 was used, how dry the plates are, and the particular serovar of *Salmonella* used. Therefore, you should always include a control of the wild-type $P22^S$ parent strain as a control.

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