Remember to put sodium citrate in the agar plates – point no. 17

E. coli Genome Manipulation by P1 Transduction

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ABSTRACT

This unit describes the procedure used to move portions of the *E. coli* genome from one genetic variant to another. Fragments of ~100 kb can be transferred by the P1 bacteriophage. The phage is first grown on a strain containing the elements to be moved, and the resulting phage lysate is used to infect a second recipient strain. The lysate will contain bacterial DNA as well as phage DNA, and genetic recombination, catalyzed by enzymes of the recipient strain, will incorporate the bacterial fragments into the recipient chromosome. *Curr. Protoc. Mol. Biol.* 79:1.17.1-1.17.8. © 2007 by John Wiley & Sons, Inc.

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INTRODUCTION

P1 transduction is a useful genetic procedure for moving selectable mutations of interest from one *E. coli* strain to another. P1 is a bacteriophage with a rather sloppy packaging mechanism, and during growth and encapsidation of its DNA into the phage head, it occasionally packages the DNA of its bacterial host rather than its own phage chromosome into the protein capsid. This property of the P1 phage means that when a P1 lysate is made, the individual particles in the lysate contain either packaged phage DNA or packaged bacterial DNA. When this lysate is used to infect a second (recipient) host, DNA pieces from the original host chromosome are transferred into the new strain, where they can recombine and be permanently incorporated into the chromosome of the second strain, an event called transduction. Successful P1 transduction of any one marker from a unique location in one bacterial strain to a second strain is a relatively rare event, so selection is required. P1 can carry two or more different genetic markers in the same transducing particle DNA, and at some frequency both of these markers can be recombined into the recipient strain. Such markers are said to be linked by P1 transduction, i.e., they are close enough to be co-transduced. Proximity of the selectable marker to the allele of interest is important, because nearer alleles will co-transduce more frequently. The allele of interest might be a (defective) gene with an antibiotic marker cassette insertion, or it might be a gene with a point mutation that is closely linked to a selectable antibiotic marker.

To execute the Basic Protocol, the P1 lysate is grown on a host containing a genetic variant of interest and this lysate is then used to infect a second strain. For the nonexpert, it is best to use a virulent phage mutant, P1*vir*, to produce the lysate because the *vir* mutation prevents the phage from generating P1 lysogens among transductants. The Basic Protocol describes growth of a liquid lysate, which is easier to carry out than a plate lysate and generally gives good results. The Support Protocol describes the procedure used to titer the P1 stock, which is usually done only if the transduction is not successful. The Alternate Protocol provides a method for preparing a plate lysate, which may be used if the starting P1 preparation has a low titer. Plate lysates often yield higher-titer stocks than liquid lysates.

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BASIC PROTOCOL

PREPARATION OF P1 LIQUID LYSATE AND P1 TRANSDUCTION

This protocol describes the two-part procedure: preparation of a liquid P1 lysate, followed by transduction. Each procedure requires a freshly grown bacterial overnight culture. Once the cultures are prepared, the procedures are simple and each requires only a few hours, so that both can be performed in the same day.

Materials

LB broth (*UNIT 1.1*)
E. coli nonlysogenic donor strain (containing the genetic marker to be transduced)
20% glucose stock solution
1 M $CaCl_2$
10^9 to 10^{10} pfu/ml P1 stock, a virulent P1 mutant preferred (e.g., P1*vir*)
Chloroform ($CHCl_3$)
E. coli recipient strain (to be transduced)
Tris-magnesium (TM) buffer: 10 mM $MgSO_4$/10 mM Tris·Cl, pH 7.4 (*APPENDIX 2*)
P1 salts solution: 10 mM $CaCl_2$/5 mM $MgSO_4$
1 M sodium citrate
Selective plates (see *UNIT 1.1*) with 5 mM sodium citrate (recommended); e.g., minimal plates if selecting for prototrophy *or* rich LB plates containing the appropriate antibiotic (depending on drug cassette used):
  30 μg/ml ampicillin
  30 μg/ml kanamycin
  10 μg/ml chloramphenicol
  12.5 μg/ml tetracycline (6.25 μg/ml if sodium citrate is added)
  50 μg/ml spectinomycin
50-ml Erlenmeyer flasks, preferably baffled
18 × 150–mm glass culture tubes
Shaking water bath or test-tube roller at appropriate temperature (usually 32° to 37°C)
35-ml plastic centrifuge tubes
Refrigerated low-speed centrifuge with Sorvall SA-600 rotor (or equivalent)
5-ml screw-cap vials

NOTE: All material coming in contact with *E. coli* must be sterile.

Prepare liquid P1 lysate

1. Inoculate 5 ml LB broth with the *E. coli* donor strain (from which the P1 lysate is to be made). Incubate overnight at the appropriate temperature.

   Throughout this protocol, the optimal temperature for cell growth is 37°C, unless a temperature-sensitive strain is used. Temperature-sensitive strains should be cultured at 30° to 32°C.

   To perform transduction on the same day, grow the recipient strain (step 9) at the same time.

2. Dilute the overnight donor culture 1:100 into 5 ml LB broth containing 50 μl 20% glucose (final concentration 0.2%) and 25 μl of 1 M $CaCl_2$ (final concentration 5 mM) in a 50-ml Erlenmeyer flask or 18 × 150–mm glass culture tube.

3. Incubate 30 to 45 min at 30° to 37°C with shaking.

   Use either a 50-ml Erlenmeyer flask in a shaking water bath or a standard culture tube with adequate aeration in a test-tube roller. If the strain is temperature sensitive, use the lower temperature and the longer time.

4. Add 100 µl of a recently prepared 10^9 to 10^{10} pfu/ml P1*vir* stock.

   A good titer for a P1 stock is 10^9 to 10^{10} pfu/ml. If the stock has recently been used successfully and does not contain $CHCl_3$ (which reduces the viability of the stock; see step 6), it is probably unnecessary to determine the titer.

5. Continue to incubate, with shaking, until the culture lyses (~3 hr).

   The culture may not lyse and clear completely, but clumps of floating debris should be visible when it is held up to the light. An unlysed culture looks smooth and silky when swirled; a lysed culture does not.

6. Add a few drops of $CHCl_3$ and continue shaking a few minutes more.

   The $CHCl_3$ ensures complete cell lysis and kills the bacteria. $CHCl_3$ does not harm the P1 phage at this step, but care should be taken to avoid transferring the $CHCl_3$ to the storage tube during step 8, because storing the phage in the presence of $CHCl_3$ can result in decreased viability of the stock over time.

7. Transfer the supernatant to a 35-ml centrifuge tube and centrifuge 10 min at ~9200 × *g*, 4°C.

8. Pour off the supernatant (the P1 lysate) into a screw-cap 5-ml tube. Label it with the strain on which the lysate was grown (e.g., P1*vir*·W3110) and the date, and store up to several years at 4°C.

   The lysate can be further purified by passage through a sterile 0.45-µm filter syringe after centrifugation. The filter will remove any residual bacteria.

   The lysate can be stored for several years at 4°C and still retain adequate titer. If in doubt, either spot titer as described in the Support Protocol or make a new lysate.

Perform P1 transduction

9. Inoculate 5 ml LB broth with the *E. coli* recipient strain. Incubate overnight at the appropriate temperature.

   If the strain does not have temperature-sensitive elements, use 37°C.

10. The following day, centrifuge 1.5 ml of the cell culture in a microcentrifuge tube 2 min at maximum speed, room temperature, to pellet the cells.

11. Discard the medium and resuspend the cells at one-half the original culture volume in sterile P1 salts solution.

    This gives 0.75 ml suspended cells, which is adequate for two transductions. Process more cells in step 10, if necessary.

12. Mix 100 µl of the cells/P1 salts mixture with varying amounts of lysate (1, 10, and 100 µl) in sterile test tubes. As a control, include a tube containing 100 µl of cells without P1 lysate.

13. Allow phage to adsorb to the cells for 30 min, either at room temperature on the benchtop or at the appropriate culture temperature. Treat the controls similarly.

    Temperature selection should take into account any temperature or cold sensitivity of the recipient strain.

14. Add 1 ml LB broth plus 200 µl of 1 M sodium citrate.

    The sodium citrate chelates the calcium essential for phage adsorption to the bacteria, thus minimizing secondary infection by residual phage.

15. Incubate ~1 hr at 30° to 37°C with aeration.

    The exact temperature will depend on the characteristics of the strain being transduced and the expected characteristics of the strain after transduction. If the desired strain is temperature sensitive, the incubation should be done at a low temperature.

I transfer to multiple 1.5ml eppendorf tubes

store in a glass bijou

next day inoculate a 5ml flask - let this grow until you have prepared the phage

in glass bijou's

16. Centrifuge each culture in a 1.5-ml microcentrifuge tube 2 min at maximum speed, room temperature, to pellet the cells. Remove and discard the supernatant.

17. Suspend the cells in 50 to 100 µl of an appropriate buffer or broth and spread the entire suspension onto the appropriate selective plate. Also spread 100 µl of the P1 stock on another plate. Incubate the plates overnight at the appropriate culture temperature.

It is good policy to supplement the selective plates with 5 mM sodium citrate, which helps prevent readsorption of residual P1 phage. If sodium citrate is used, one-half the concentration of tetracycline must be used.

don't forget this bit!

Antibiotic resistance is a commonly selected marker: in this case, LB broth can be used for dilution. Be sure to use plates with the lower drug concentrations needed for single-copy genes. Prototrophy can also be selected: in this case, the cells should be washed and diluted in a minimal salts medium such as TM buffer.

The P1-only control is used to determine whether contaminating bacteria are present in the lysate.

18. The next day score the plates.

The control plates with cells only or P1 phage only should be free of bacterial colonies; the other plates should contain the transduced colonies. Usually the plate receiving the lowest amount of P1 lysate will have the fewest colonies.

If colonies are present on the cells-only control plate, either the selection is not working or contamination is present.

If colonies are present on the phage-only plate, the P1 lysate is likely contaminated with donor bacteria. This should not occur if the $CHCl_3$ treatment was complete. If this control gives colonies, filter the lysate through a sterile 0.45-µm filter and try again.

19. From the plate that shows growth from the least amount of P1 phage, pick single colonies, streak to isolate single clones, and incubate plates overnight at 30° to 37°C. Repeat this process a second time.

Picking colonies from the plate to which the least amount of P1 was added is good microbial practice because many infective P1 phage particles will remain on the plates. Two rounds of purification are essential to ensure removal of such residual P1 phage and separation of nontransduced bacteria from the actual transductants.

Table 1.17.1 Correlation of Linkage with Physical Distance for P1 Transduction

Percentage linkage	Physical distance (kb)
0	—
2	67.03
10	49.30
20	38.20
30	30.41
40	24.21
50	18.98
60	14.40
70	10.31
80	6.59
90	3.17
100	0.00

If the genetic marker selected is identical to the mutation of interest (100% linkage), every colony should express the mutant phenotype. If the genetic marker selected is not tightly linked to the mutation, more colonies must be screened for the desired phenotype (see Table 1.17.1).

20. Confirm the genotype of the purified transductants by drug-resistance or phenotype.

    If there is no genetic way to score the desired mutation, use PCR to amplify the region of interest (using standard primers) and sequence the PCR product.

TITRATION OF THE P1 LYSATE

SUPPORT PROTOCOL

Although P1 transduction is usually performed without titering the phage lysate, the phage should be assayed if the transduction is not successful. This protocol describes spot titering, where the lysate is serially diluted and applied to a bacterial lawn. Spot titering conserves materials and may facilitate visualization of the P1 plaques, which are very tiny.

Additional materials *(also see Basic Protocol)*

Standard *E. coli* K12 strain (e.g., C600 or MG1655)
LB plates and top agar (*UNIT 1.1*), each containing 2.5 mM $CaCl_2$

1. Inoculate 5 ml LB broth with a standard *E. coli* K12 strain. Incubate overnight until cells reach mid-log phase ($\sim 2 \times 10^8$ cells/ml).
2. Add 1 M $CaCl_2$ to a final concentration of 5 mM.
3. While the cells are growing, make serial ten-fold dilutions (through 10^{-8}) of the phage stock in TM buffer.
4. Pour a lawn of the cells on an LB/$CaCl_2$ plate by mixing 0.25 ml of the cell culture with 2.5 ml LB top agar (with $CaCl_2$) in a small culture tube and immediately pouring the contents onto the plate. Tilt the plate gently to distribute the cell-agar mixture uniformly across the surface of the plate.
5. After the agar hardens, spot 10 µl of each phage stock dilution onto the lawn of cells. Let the spots dry and then incubate the plate upright at 37°C.

   After overnight incubation, individual countable plaques should be present in one of the dilutions.

6. Calculate the titer of infective phage particles/ml (pfu/ml) by counting the number of plaques and multiplying by the fold dilution and a factor of 100 (to account for the 10 µl volume spotted).

   If the only P1 lysate available has a poor titer ($<10^9$ pfu/ml), a new high-titer stock must be grown. In this case, use a confluent 10-µl spot of phage to inoculate a liquid culture and make a high-titer liquid lysate (see Basic Protocol, steps 1 to 8). The spot can be taken from the lawn of cells using a sterile spatula (do not dig into the bottom agar).

PREPARATION OF P1 PLATE LYSATES

ALTERNATE PROTOCOL

Plate stocks can give higher titers than liquid stocks, although they are often not necessary. This is a standard procedure for making P1 plate lysates. Variations exist and may also work well. For materials, see Basic Protocol.

1. Inoculate 5 ml LB broth with the *E. coli* donor strain. Incubate overnight at the appropriate temperature.

   The optimal temperature is 37°C, unless a temperature-sensitive strain is used. Temperature-sensitive strains should be cultured at 30° to 32°C.