**P1 TRANSDUCTION**
(adapted from initial protocol by Dr. Brad Weart-Levin lab)
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2008

**Step 1: Grow phage on recipient strain**

In this step the phage is grown on the strain of bacteria that will be receiving the genetic modification. This is just a preliminary step to ensure that the procedure is as clean as possible (i.e., to reduce the risk of transferring foreign DNA).

**Materials**
LB + 5 mM CaCl$_2$ + 0.2% glucose
chloroform

1. Streak for single colonies of recipient strain.
2. Inoculate liquid culture (LB broth) with single colony.
   a. Liquid culture: LB + 5 mM CaCl$_2$ + 0.2% glucose (for 5 mL LB add 25 uL CaCl$_2$ (1 M stock) and 50 uL glucose (20% stock)). Calcium facilitates the physical interaction between the phage and the bacterial cell.
3. Grow to OD$_{600}$ of 0.1-0.2 (takes between 1 and 2 hours). If the culture grows past this OD, dilute back to OD$_{600}$ of 0.1 and then shake for 15-20 minutes before the next step.
4. Add 100 uL phage stock and shake at 37 C for 3 hours.
5. Pull culture and add 3 drops of chloroform per mL of culture (for 5 mL culture add 15 drops) to kill the recipient cells. Vortex briefly.
6. Transfer culture to centrifuge-safe tube and spin for 5 min at 5000 rpm.
7. Remove supernatant to fresh tube, add 3 drops of chloroform per mL of culture, vortex briefly, and store at 4 C. The phage generated in this step will be the phage stock for step 2. The pellet will be very loose after the spin, so be careful when removing the supernatant. It is okay if not all of the supernatant can be removed—only a small volume of phage is necessary for the procedure.

**Step 2: Grow phage on donor strain**

In this step, the phage from step 1 is grown on the bacterial strain whose genetic modification will be transferred. This step is identical to step 1 with the exception of the strain—here the donor strain culture is inoculated with phage.

1. Repeat step 1, using donor strain instead of recipient strain.

**Step 3: Transfer genetic modification to recipient strain**

In this step, the phage (carrying the genetic modification from the donor strain in step 2) will be
Materials

- LB + 5 mM CaCl$_2$ + 0.2% glucose
- LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$
- 1 M NaCitrate pH 5.5
- LB + 100 mM NaCitrate
- LB plate + antibiotic

1. Streak for single colonies of recipient strain.
2. Inoculate liquid culture with single colony.
   a. Liquid culture: LB + 5 mM CaCl$_2$ + 0.2% glucose (for 5 mL LB add 25 uL CaCl$_2$ (1 M stock) and 50 uL glucose (20% stock)).
3. Grow to OD$_{600}$ of 0.8-1.0 (takes about 3 hours). If the culture grows past this OD, dilute back to OD$_{600}$ of 0.8 and then shake for 15-20 minutes before the next step.
4. Transfer culture to centrifuge-safe tube and spin for 5 min at 5000 rpm.
5. Resuspend the cell pellet in ¼ volume of LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$ (assuming a 5 mL initial volume, and assuming 1 mL will be removed for the OD$_{600}$ reading, pellet should be resuspended in 1 mL LB + 5 uL CaCl$_2$ + 100 uL MgSO$_4$).
6. Set up four reactions in 1.5 mL Eppendorf tubes:
   a. 100 uL recipient cells with 100 uL undiluted phage
   b. 100 uL recipient cells with 100 uL 1/10 dilution of phage in LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$ (10 uL phage + 90 uL LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$).
   c. 100 uL recipient cells with 100 uL LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$ (control for spontaneous resistance)
   d. 100 uL phage with 100 uL LB + 5 mM CaCl$_2$ + 100 mM MgSO$_4$ (control for presence of any unkill donor cells)
7. Incubate at 37 C without shaking for 30 min
8. Add 200 uL 1 M NaCitrate pH 5.5 to the Eppendorf tube and mix. NaCitrate is a calcium chelator that is used to disrupt the physical interaction between the phage and the bacterial cell.
9. Add 1 mL LB to a 4 mL glass culture tube, then transfer the reaction mixture plus NaCitrate from the 1.5 mL Eppendorf to the 1 mL LB.
10. Shake for 1 hour at 37 C. Making sure that the culture gets enough aeration in this step is crucial to the efficiency of the transduction, so put the culture in a good shaker!
11. Pull culture and spin for 1 minute at 13,000 rpm.
12. Discard supernatant and resuspend pellet in 100 uL LB + 100 mM NaCitrate.
13. Plate 100 uL to LB + antibiotic and incubate at 37 C overnight.
Step 4: Isolating transductants

The following day several colonies should be visible on the test plates (plates with reaction mixture “a” and “b”). If there are no colonies 24-48 hours after plating, the transduction did not work and should be repeated. Typically, spontaneous antibiotic resistant mutants appear on the first control plate (reaction mixture “c”) after 48 hours and are very small. If colonies appear on the second control plate (reaction mixture “d”) then your phage stock could be contaminated with cells from the recipient strain.

Materials
100 mM NaCitrate
LB plate + antibiotic

1. Take out the LB plate + antibiotic about one hour before you plan on using it.
2. Add 200 uL of 100 mM NaCitrate pH 5.5 to LB plate + antibiotic and spread.
3. Place upright at 37 C to dry for one hour.
4. Pick single transductant, streak, and incubate plate overnight at 37 C.

Step 5: Initial verification of transductants

If there is some physiological screen, such as lac +/-, then this is a good initial screen for correct transductants on X-gal plates.

Step 6: PCR verification of transductants

1. Extract genomic DNA from transductants (see extraction protocol)
2. Verification PCR
   For the verification of the correct genetic modification, one primer will need to recognize a region of DNA outside of the modification on the genome of the recipient, and the other primer will need to recognize the genetic modification itself. For the Keio strains, the second primer will recognize the kanamycin resistance cassette. Pay attention to which primers are forward and which are reverse.
   a. Run PCR.
   b. Pour 1% agarose gel + 8 uL ethidium bromide. Load 5 uL PCR reaction + 5 uL ddH₂O (10 uL total) and run gel at 100 V for 30-45 min
   c. Take picture of gel. The presence of a correctly-sized product indicates a successful transductant, while the absence of a PCR product likely indicates a false transductant.