Constructing *E. coli* strains by P1 transduction

<u>Lysate preparation</u>:

1. Streak donor (contains gene knock-out or modification of interest) and recipient strains onto agar plates the night before. (remember the recombineering strain is temperature sensitive!).
2. Start donor strain culture in the morning. Pick single colony and grow in 8 ml LB + 5 mM $CaCl_2$ between 6 and 18 hours. This may take longer if you're working with a strain that's sickly or is temperature-sensitive (i.e. requiring growth at 30C). You're looking for a culture that's fairly dense.
3. Before beginning the infection, melt R top-agar and keep it at 42C. Also, warm LB agar plates in the 37C oven.
4. Aliquot 900 μl of culture into four tubes. Infect first tube with 100 μl of P1 virion lysate. Flick gently to mix. Do two serial 10-fold dilutions, i.e. transfer 100 μl of first tube's contents into a second 900 μl tube of culture and then 100 μl of second tube's contents into the third. Keep the forth tube as an uninnoculated control. Be sure to change pipette tips between aliquots. Incubate these mixtures in a 37C (or 30C for temperature-sensitive strains) water bath, 30 to 40 minutes, without shaking.
5. At the end of incubation, add 4 ml top-agar to each tube. Flame test tube gently and pour mix onto LB plate. Swirl plate gently to distribute mixture. Incubate overnight at 37C (or 30C for ts strains).
6. Using a glass pasteur pipet melted to form a scraper, remove top-agar from plates where P1 phage has lysed most, if not all of the cells. Do NOT take plates that have small numbers of plaques scattered over a lawn. Acceptable plates are those which either have a high density of overlapping plaques that mask the lawn of bacteria below or which have clear fields marked by clumps of cell debris. Note, P1 phage makes small pin-prick plaques.
7. Collect agar into Eppendorf tubes. Centrifuge 15 minutes at 14,000 rpm. Remove supernatant and add 100 ml chloroform, and spin it again for 15 minutes at 14,000 rpm. Collect supernatant, taking care to avoid interface (leave some supe behind). Add a few drops of chloroform and store at 4C.

Transduction:

1. Start a culture of the recipient strain (from a single colony) in 8 ml LB + 5 mM $CaCl_2$. Grow at 37C (or 30C for ts strains) for 6 hours to overnight.
2. Pellet 1 ml of cells per 8 transduction reactions, 45 seconds at 14,000 rpm. Resuspend each pellet in 800 ml MC buffer (5 mM $CaCl_2$, 10 mM $MgCl_2$).
3. Right before use, spin down your P1 lysate (about 350 ml for a full set of reactions: 2 dilutions and controls) for 5 minutes at 14,000 rpm.
4. Set up transductions as follows:

Tube 1	100 ml LB	100 ml cells	100 ml P1 lysate
Tube 2	100 ml LB	100 ml cells	10 ml P1 lysate
Control 1	100 ml LB	100 ml cells	NO lysate
Control 2	100 ml LB	NO cells	100 ml lysate

5. Incubate 15 to 20 minutes at 30C. Don't let infection proceed for too long, lest the cells all lyse and then reinfect your transductants, leaving you with no colonies.
6. While infection is proceeding, warm and "dry" selective agar plates at 37C by lifting lid off each plate.
7. At the end of the incubation, add 40 ml 1M sodium citrate to each tube, vortex and pellet cells for 45 seconds at 14,000 rpm. Resuspend each cell pellet in 100 ml of LB + 0.1M sodium citrate.
8. Spread onto plates until mixture is completely absorbed, and the plate looks dry. Incubate at 37C (or 30C for ts strains) overnight or up to 48 hours.
9. Streak any transductants onto selective plates.
10. Pick single colonies and grow overnight cultures for freezing and to check for strain markers, e.g. thy +/-, uv sensitivity, etc, and lysogeny.

Drug concentrations to use are as follows:

Amp	100 mg/ml
Tet	15 mg/ml
Cam	20 mg/ml
Kan	30 mg/ml
Spc	20 mg/ml