Using P1 Transduction to construct new recombinant strains

Procedures:

Lysate:

1. Grow Donor Strain (hyper-recombinant priB and priC knockouts strains) overnight in LB + 5mm CaCl2.
2. Add 100ul, 10ul, and 1ul of P1 lysate (solution containing the phage) to 3, 1ml tubes of your donor culture. Incubate 37C, 30min.
3. Add 4mls of top agar (43C) and pour onto LB plates. Let agar solidify. Incubate 37 overnight.
4. Scrape top agar from the most dilute plate that lysed almost all the cells. (Plaques on more dilute plates should look VERY small, round and clear.)
5. Centrifuge 20 min top 14000 rpm. Take supernatant, add a few drop of chloroform, spin again. Take supernatant, add a few drops of chloroform. Store at 4C.

Transduction

1. Grow Recipient strain (SR108) overnight in LB + 5mm CaCl2.
2. Pellet 1 ml of recipient cells and resuspend in MC buffer (5mm CaCl2 10 mm MgCl2)
3. Then for each cell line and P1, add the following: (spin down P1 lysate immediately before using for 5min.

Tube 1	100ul LB	100ul cells	10ul P1
Tube 2	100ul LB	100ul cells	100ul P1
Tube 3	100ul LB	100ul cells	NO P1
Tube 4	100ul LB	NO cells	100ul P1

4. Incubate 20 min at 30C.
5. Add 40ul 1M Sodium Citrate to each tube, vortex, pellet cells, resuspend in 100 ul of LB+0.1M Sodium Citrate.
6. Spread on selective plates. (In this case, CAM thy LB)
7. Incubate at least overnight 37C.
8. Day2. Restreak transductants onto selective plates.
9. Day3. Grow overnight cultures of transductant to freeze and to check for any markers that will identify the transductant as unique from either the donor or the recipient.