Caulobacter phage transduction

Preparing phage lysates:

1. Grow donor strain overnight in PYE
2. Prepare PYE top agar (0.3% agar in PYE, 5 ml per lysate). After autoclaving, place flask in 45°C water bath.
3. Add 1 µl ΦCr30 phage to 100 µl of donor cells
4. Incubate at 30°C for 15 min
5. Add cells to 4 ml of PYE top agar and pour mixture onto a pre-warmed PYE plate
6. Allow the agar to solidify and grow overnight (right-side-up) at 30°C. The following morning, the plate should have confluent lysis (many phage plaques on the plate).
7. Scrape the top-agar into a 50 ml conical tube
8. Wash the plate with 5 ml PYE and add to the same conical tube
9. Add 100 µl chloroform to the tube and vortex to kill remaining cells
10. Incubate at room temperature for 1 hour
11. Vortex again and centrifuge for 10 minutes at 3,400 x g
12. Transfer supernatant to a petri dish and irradiate for 1 minute with no lid
13. Transfer to a clean 15 ml conical tube and add 100 µl chloroform
14. Store phage at 4°C

Transduction:

1. Grown recipient strain overnight in PYE
2. Mix 500 µl culture with 475 µl PYE and 25 µl phage-lysate
3. Incubate at 30°C for 1 hour
4. Collect cells by centrifugation and plate on selective media