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Conjugation Protocol for Marine Strains

This protocol assumes you have made 24-well plates with medium + 0.3mM DAP (~1-2 ml per well) and have characterized the natural antibiotic resistance of the recipient strains. Conjugation is a reliable method to introduce a plasmid into strains of interest. Protocol was adapted from the Barrick Lab, Septer Lab, and Schwartzman Lab conjugation protocols.

1. Start an overnight culture of donor and recipient strains. Donor strain is *E. coli* RHO3+ which is a DAP auxotroph – inoculate it into LB24 +0.3mM DAP + antibiotic. Recipient strains can be grown up in Marine Broth 2216, ½ Marine Broth 2216, ½ YTSS, or whatever you have.
2. The next morning, record the OD of each culture.

3. Adjust cultures so they are roughly the same OD (fine if they aren't exact)
4. Calculate the volume of donor strain needed to do all conjugations. (300 ul per conjugation at 3 ratios)
5. Spin down the 500 ul culture (10000 x g, 5 min). Wash cells thrice in PBS or in sterile media. This will remove residual antibiotic from the donor strain. *E. coli* does not seem to pellet well in Marine Broth, monitor pellet.
6. Mix Donor:Recipient in ratio of 1:1, 1:10, 10:1 in a total of 200 ul (can use other ratios if needed).
7. Spin the mixture and remove all but ~10 – 20 ul of medium, resuspend pellet.
8. Spot the whole volume into 1 well of a 24-well plate filled with MB + 0.3mM DAP (WITHOUT ANTIBIOTIC). Let spot dry and incubate for 24 – 48 h.
9. Either scrape up conjugation mixtures that look promising (if fluorescent plasmids, they may have color to them) and transfer to 500ul of PBS or media, or pipette 500 ul into conjugation mixture and pipette up and down to gather cells.
10. Vortex and gently spin down, wash cells, and repeat.
11. Plate 100ul of this mixture onto antibiotic selective plates (WITHOUT DAP).
12. Pick single colonies and re-streak on selective media. Confirm transfer of the plasmid via PCR amplification.