Mating Protocol

Preliminary calculations:

Want 250μl of OD600=10 per filter, or 2.5 OD's/filter for EACH *E.coli* strain.

Want 500μl of 100μg/ml chla for each filter, or 50μg Chla/filter.

1. Have 500ml exponential ammonia grown Nostoc ready. Take chla reading and harvest enough for all matings. Resuspend to 100μg chla/ml and use 500μl per filter.

2. Inoculate 1/40th overnight culture of eacn E.coli strain into selective LB. Check OD600 and use when near 1. Calculate total OD600 units per culture. Centrifuge and resuspend to OD=10. Mix E. coli strains 1:1, to allow for 500μl per mating filter. Centrifuge and washtwice in LB.

3. Wash Millapore HATF nirocellulose filters in d water and place between Watman #1 filters. Autoclave in glass petri dish dry cycle(20-20min). Place atop AA mops, ammonia, 0.5% LB mating plates.

4. Mix 500μl each *Nostoc* and *E.coli* from step 1&2 in a microfuge tube and spin briefly in microfuge. Pipet off all but ~200-300μl of supernatant and resuspend by pipetting up and down. Place atop mating filter (step 3) and spread. Allow for moisture to be absorbed/dried before parafilming.

5. Place in low light (bottom shelf CO2 incubator) overnight. Tansfer filter to AA mops ammonia and allow to recover in low light. When cells look healthy transfer filter to AA,mops ammonia, antibiotic plates, and place in high light