Rhodobacter Conjugative Transformation Procedure

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Bacterial conjugative transformation of transferring recombinant plasmids into *Rhodobacter capsulatus*:

Heat shock transformation of plasmid into Chemically Competent *E. coli* S17-1:

1. Thaw eppendorf tubes containing chemically competent *E. coli* S17-1 cells on ice. Add 1 uL (approx 100 to 500 ng) of plasmid solution in S17 cells. Leave cells to thaw on ice for 30 minutes.
2. Heat shock at 42°C in a water bath for 45 seconds and immediately place back to ice for additional 5 minutes.
3. Recover cells with 0.5 mL of SOC medium.
4. Incubate in 37°C shaker (200 rpm) for 1 hour
5. Dispense 100 uL of the culture onto plate (with selection).
6. Incubate at 37°C overnight or until colony formation.

Cryostock preparation of each plasmid vector to be stored at -80C:

1. After cells grow on plate, take single colony into test tube/15 mL falcon tube of 2-3 mL of LB (with selection).
2. Incubate at 34°C shaker overnight.
3. Take 1 mL of culture and centrifuge at 4000 RCF for 2 minutes.
4. Discard supernatant.
5. Add 500 uL of LB media and 500 uL of sterile 50% glycerol. Mix well by pipetting up and down to resuspend all cells. Transfer mixture into sterile cryo-tube and drop into liquid N_2.
6. Collect tubes from liquid N_2 and transfer to -80°C.

Conjugation of *R. capsulatus* with *E. coli* S17-1 (carrying antibiotic resistant plasmid):

Growing cultures:

1. Streak from *R. capsulatus* cryostock onto YCC and incubate at 34°C for 24 hours or until colony formation.
2. Streak from *E. coli* S17-1 cryostock onto LB plate with selection and incubate at 37°C overnight or until colony formation.
3. Grow cultures of recipient strain *Rhodobacter capsulatus* by transferring single cell colony from plate to 25mm culture tube containing 4 mL of YCC and place in 34°C shaker for 1-2 days. Timing is not critical since culture will be most likely in its stationary phase.
4. Grow cultures of donor strain *E. coli* S17-1 by transferring single cell colony from plate to culture tube containing 2-3 mL of LB+Kanamycin and place in 37°C shaker for ~7-9 hours (OD_{600} 0.5-0.6). Timing is critical to have culture still in its exponential phase. Alternative is to grow donor strain for ~12 hours or in its stationary phase then restart cultures approximately 6 hours before conjugation.

Mating:

1. Measure and record OD_{660} (0.2ml+0.8mL dd H_2O; tap water blank) of *R. capsulatus* and OD_{600} (0.2ml+0.8mL dd H_2O; tap water blank) of S17.
2. Transfer the following into separate eppendorf tubes:
   - 0.5 mL of donor *E. coli* S17-1
   - 0.5 mL of recipient *R. capsulatus*
3. Centrifuge at 12000 RPM for 5 minutes and decant the supernatant of both.
4. Resuspend *E. coli* and *R. capsulatus* in 0.5 mL YCC. Resuspend, re-centrifuge at 12000

RPM for another 5 minutes, and decant the supernatant of both.
5. Resuspend *E. coli* in 200 uL YCC.
6. Resuspend recipient *R. capsulatus* in 200 uL YCC.
7. Combine 50 uL of *E. coli* and 50 uL of *R. capsulatus* and dispense 100 uL mating mixture onto sterile nitrocellulose membrane filters (0.45 or 0.025 uM diam) that have already been placed on YCC plates. Allow spots to dry (20 minutes) by diffusing into agar then incubate at 34°C with filters facing upwards for 1-2 days.

Elution and Plating:
1. With sterile tweezers, pull filter off plate and place in eppendorf tube containing 1 mL of YCC. Allow filter to soak for 5 minutes.
2. Pipet up and down to resuspend all cells. Dispend 100 uL onto selective YCC plates. Incubate plate at 34°C overnight/until colony forms.
3. Check plates for colonies and restreak up to 6 colonies onto YCC with selection to verify no presence of *E. coli*. Place plate in 34°C incubator for 24 hours. (can also confirm by PCR).
4. If plasmids are confirmed to be successfully transformed into *R. capsulatus* (by restreaking and colony PCR), transfer colonies to liquid broth and make cryostock using the stock solution of 50% glycerol to a final concentration of 25%.