

Shewanella Conjugation with *E.coli* WM 3064

Cornell iGEM 2012 Protocol

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Adapted from email correspondence with Dr. Jeff Gralnick.

Requirements before beginning protocol: Before setting up conjugation, you must have a fresh plate of WM 3064 transformants (with plasmid of interest), obtained by electroporating a 50 μL competent freezer stock with no more than 10 ng and no less than 10 pg plasmid. Ideally, plasmid should be at a concentration of 10ng/ μL , so that 1 μL DNA can be added to competent cells. Adding larger volumes will decrease transformation efficiency due to increased salt content. After electroporation, cells should be allowed to recover in about 900 μL SOC for one hour in a 37 ° C in a shake incubator, then spun down and resuspended in about 200 μL SOC before plating on a DAP (0.3mM) + antibiotic LB agar plate. SOB or LB will work if SOC is unavailable.

Day 1 (Evening)

1. Grow an overnight culture of the donor (WM3064 + plasmid) with 1 mL LB, 12 μL 100x DAP stock solution, and 1 μL 1000x antibiotic, as appropriate.
2. Grow overnight culture of the *Shewanella* recipient (typically JG 700) in 1 mL LB without antibiotic.

Day 2

1. After about 14 hours, spin down 250 μL WM 3064 culture, wash once with 500 μL LB, and resuspend the pellet with 150 μL of the *Shewanella* culture.
2. Add entire volume of cells to an LB DAP plate (**without antibiotic**). Swirl the cells around a bit; the entire plate doesn't need to be covered. Place plate under a flame with the lid ajar to wait for the surface to dry before flipping the plate over.
3. Incubate at 30 degrees for about 8 hours.
4. Using a sterile stick, take a large wad of these cells and streak for single colonies on a LB + antibiotic plate (**without DAP!**).
5. Incubate at 30 degrees for about 12-16 hours.
6. Restreak a few colonies to new LB + antibiotic reference plates while making overnight cultures from same colonies.
7. Miniprep from overnight cultures (using modified *Shewanella* miniprep protocol), quantify, and submit for sequencing to confirm successful conjugation.