

Caulobacter Conjugation Protocol

(Klein, June 2016)

1. Grow donor *E. coli* strain overnight in LB with selection antibiotic (0.1 ml per conjugation)
2. Grow recipient *Caulobacter* strain overnight in PYE (1 ml per conjugation)
3. Pre-warm a PYE agar plate at 30°C
4. Mix 1 ml *Caulobacter* with 100 µl *E. coli* in a microcentrifuge tube and immediately collect cells at 8,000 RPM for 2 minutes
5. Resuspend cell pellet in 10 µl PYE and drop the mixture onto the PYE agar plate (20 µl drop)
6. Once the droplet has been absorbed into the plate, incubate the plate overnight at 30°C.
7. Use a wooden stick to streak out the conjugation mixture onto PYE plates containing the appropriate plasmid/cosmid antibiotic selection as well as nalidixic acid (20 µg/ml) to kill the *E. coli*
8. Incubate plates at 30°C for 2-3 days until colonies appear