

## Caulobacter Transformation Protocol

(Klein, September 2015)

1. Grow recipient strain overnight in PYE (3 ml culture per transformation)
2. Pre-chill 1 mm electroporation cuvette (white cap)
3. Split 3 ml culture into two 1.5 ml eppendorf tubes and centrifuge for 2 min at 8000 rpm at 4°C
4. Keep tubes on ice for remainder of protocol
5. Decant supernatants. Wash and combine pellets in 1 ml cold-sterile water
6. Centrifuge for 2 min at 8000 rpm at 4°C
7. Repeat steps 4-5 once more
8. Resuspend pellet in a total of 100 µl water

For integrating plasmids:

1. Add 10 µl of miniprep plasmid to bacteria
2. Pipette all 110 µl into cuvette
3. Pulse on EC1 setting
4. Recover in 1 ml PYE for 1 hr at 30°C
5. Centrifuge for 2 min at 8000 rpm, resuspend cells in 200 µl PYE, and plate entire transformation on selection plates

For replicating plasmids:

1. Add 10 µl of miniprep plasmid to bacteria
2. Pipette all 110 µl into cuvette
3. Pulse on EC1 setting
4. Recover in 1 ml PYE for 1 hr at 30°C
5. Place a 10 µl drop of transformation onto selective plates and streak for single colonies