

Transformation procedure:

Have TB and PEG at room temperature.

Have centrifuge and rotor at room temperature.

Have DNA (5 µg) in TE in anaerobic hood at least 2 hr prior to transformation. Max volume 50 µl.

Grow culture in McC to late growth phase, OD₆₀₀ between 0.7 and 1.0. Measure OD using water as blank. Make sure culture is well fed (shaking at 37°C under pressure w H₂-CO₂) and happy. To increase the chance that the culture will be at the right stage in the morning, it is a good idea when starting cultures to inoculate with a drop or two, then make 1:5 and 1:25 dilutions.

Pressurize to 30 psi H₂-CO₂.

Spin cells at room temperature at 1070 g (2500 rpm in FiberLite rotor [F13-14x50cy ml]) for 10 min

Remove supt by inverting tube and inserting needle through stopper such that its point ends up at the very edge of the stopper inside the tube. Allow pressure to push out all the supt, holding tube at such an angle that the last drop gets out.

Add 5 ml TB and thoroughly resuspend pellet by tapping tube.

Repressurize with 30 psi H₂-CO₂

Spin 10 min at 1070 g (2500 rpm) and remove supt.

Resuspend in 0.375 ml TB

Take into hood, remove stopper, and add DNA. Immediately mix. Restopper and take out of hood.

Flush with 100% N₂ 1 min. Do not pressurize yet.

Carefully add 0.225 ml PEG anaerobically with syringe holding tube so that PEG falls in drops to bottom of tube. Mix thoroughly.

Pressurize to 30 psi with 100% N₂.

Incubate at 37° 1 hr w/o shaking.

Have ready a tube of McC that has had sulfide added to it and is under pressure. Insert the long end of a vacutainer needle into transformation tube and let pressure come out. Just as the pressure is fully released, push inverted tube of McC onto short end of vacutainer needle and let medium flow into transformation tube. Mix.

Repressurize w H₂-CO₂, then spin 10 min at 1070 g (2500 rpm).

Have ready another tube of McC w sulfide under pressure. Remove supt by inserting long end of a vacutainer needle. After supt is removed and pressure is just released, push tube of McC onto other end of vacutainer needle, invert, and let medium transfer in. Resuspend pellet.

Flush w H₂-CO₂, pressurize to 40 psi, shake at 37° 4 hr to o/n, plate out.