Standard inoculation conditions - Rosanne Hertzberger, October 2017, update February 2019

I use 20% glycerol stocks. We do not have dry ice so I use regular ice boxes to transfer the tubes from the -80 to the bench and back, which means they become quite a bit warmer/softer compared to using dry ice. With disposable inoculation loop, I transfer a small scoop of material and spread it out over an MRS agar plate. These plates are transferred to an Oxoid transparent jar. After closing the jar I apply three cycles of: pulling a vacuum and filling with N_2+5%CO_2 gas. I incubate at 37 degrees.

The plates must be used between 48 hours and 7 days. Plates that were left anaerobically at room temperature were found to not lead to good precultures after 9 days. To inoculate a liquid preculture I add a “scoop” from these plates to 2 mL of NYC glucose medium in 12 mL Greiner tubes (ref 164162). I close the tubes of with the caps but do not screw them tight to leave air flow to allow for the liquid to equilibrate with the anaerobic atmosphere. Single colony has not given good results previously.

I regularly let the preculture incubate for 4 days (96 hours), inoculate all strains on Thursday and use them on the next Monday. This is twice as long as the recommended growth times of the slowest growing bacteria in my practice (*L. iners* DSM strain). Optimally, I would use 48 hours however, this is impractical due to the experiments I am running and the time I have. Furthermore, I have not experienced any problems using this preculturing time.

(Previously I have also inoculated directly into liquid culture, 2 mL of standard NYCIII glucose medium (see protocol) and regularly checking for homogeneous colony morphology on plates.)

Standard glycogen growth assay

I have used NYCIII medium supplemented with either 0.5% glycogen or nothing (water, negative control) or 0.5% glucose as a positive control. To this end I use deepwell 96 well plates (individually wrapped, sterilized) have pipetted 100 uL of a 5% glucose solution (in water) or 5% glycogen solution (in water) or water and 900 uL of a 1.1x NYCIII medium where I have left out the glucose (see separate protocol for medium). I have inoculated this with 100 uL of a preculture (see above). For every condition, I use three wells as technical replicates. In some growth experiments I have used two technical replicates for water. I also include empty controls without cells.

The plates are covered with lids that allow for gas flow at the edges. I incubate the plates anaerobically, without shaking, at 37ºC, in the Oxoid jar as described above. After 48 hours I mix by pipetting up and down with a 1 mL pipet and dilute the culture 10x with PBS in a flatbottom 96-well plate to measure the cell density in the plate reader at OD600.

MRS agar plate

Dissolve 51 gram of Sigma-Aldrich MRS broth (nr. 69966) in 1L of water, add 1 gram/L Tween80. stir until dissolved. Add 10 g/L agar and autoclave at 121°C for 20 minutes. Poor ~20 mL sterilely into petri dishes in the flow cabinet. Let them solidify and dry. Store at 4°C.