inoculate both BY4743 and FY4 TOR1Δ0 in 5 mL Carbon limiting and 5mL Leucine limiting media and grown overnight at 30 °C
dilute to 7.5x106 cells in 350 mL fresh media

Density Fractionation:
- dilute Percoll 9:1 (vol/vol) with 1.5 M NaCl to a final concentration of 167 mM.
- to form the gradients add 10 ml of the Percoll solution in a 15 mL tubes and centrifuge at 13,800 RPM for 15 minutes at 20°C.
- take cell count and pellet 2x109 cells (200 OD600) and resuspend in 1 ml Tris buffer (15 mM Tris-HCl, pH 7.5) and overlay onto the preformed gradient
- centrifuge at 400 g for 60 min in a tabletop centrifuge at 20°C
- wash fractions in a 40 ml Tris buffer, pellet, and resuspend in distilled water.
- the fractionation of density standards was performed similarly using density marker beads

Glycogen and Trehalose Assays:
- collect and pellet cell samples in parallel with those used for density fractionations and quickly wash with 1 mL of ice-cold water
- collect 20 OD total cells and resuspend in 1.0 mL of 0.25 M Na2CO3 and store at -80°C until processed
- transfer 0.5 mL of the cell suspension into two capped Eppendorf tubes (one tube for glycogen assay and the other tube for trehalose assay)
- boil cell samples in 0.25 M Na2CO3 for 4 hours
- add 0.15 mL of 1 M acetic acid and 0.6 mL of 0.2 M sodium acetate to each sample
- transfer half of each sample to another Eppendorf tube as a control
- transfer the the remaining half of the sample was incubated overnight with:
  - 1 U/ml amylglucosidase rotating at 57°C for the glycogen assay
  - 0.025 U/ml trehalase at 37°C for the trehalose assay
- centrifuge samples at top speed for 3 min
- use Glucose Assay kit to assay for the glucose liberated in a 96-well plate format
- add samples into each well (with or without dilution with water) to fit into the linear concentration range of the assay (20–80 µg/ml) to a total volume of 40 µL per well
- preincubate the plate at 37°C for 5 minutes
- add 80 µL of the assay reagent from the kit to each well to start the colorimetric reaction
- after 30 minutes of incubation at 37°C, add 80 µL of 6 M H2SO4 to stop the reaction
- determine absorbance at 540 nm to assess the quantity of glucose liberated from glycogen and trehalose