Density Fractionation and Trehalose,Glycogen Assay

**Day 1:** inoculate strains in 3 mL Leucine limiting media and incubate overnight at 30°C

**Day 2:** back dilute into 76 mL fresh media

**Day 3:** back dilute to 5x10^6 cells/mL in 65 mL fresh media and collect saturated sample from previous overnight

For each time point: 10 mL for trehalose assay, 10 mL for glycogen assay, 50 mL for density fractionation

**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 10,000 RPM for 20 minutes at 20°C.
- take cell count and pellet 2x10^9 cells (200 OD600) and resuspend in 1 ml Tris buffer (50 mM Tris-HCl, pH 7.5) and overlay onto the preformed gradient
- centrifuge at 500 RPM at 20 minute intervals in a fixed rotor.
- wash fractions in a 40 ml Tris buffer, pellet, and resuspend in distilled water.

**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 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 amyloglucosidase 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 complete assay

**Glucose Assay**