

Lab Notebook Entry 36

Date Performed

5/25/17

Procedure

transformation of pThermolyse backbone (we think...) into subcloning cells

Details

Purpose: get started making more of our backbone, so that if the sequencing shows that our gibson assembly was successful we can proceed and not have lost the two days it took to sequence. if it comes back with a bad sequence, we will toss this and start over. Materials: Subcloning chemically competent cells (KEEP ON ICE!), pThermolyse backbone miniprep products, Six LB-amp plates (if fewer than six, use what is there, and prioritize plating 500uL for each transformation before you plate 10uL, also let Aaron know that we are out), water bath (get it up to 42C when you're setting up), shaking incubator, SOC media, sterile disposable spreader, foam floater for water bath

Protocol

Use protocol in 2017 file. You are going to be doing 3 transformations, one with each of the three DNA samples we have from gibson assembly. Each of the transformations will be plated in two different concentrations on two plates. Label the plates "pThermolyse backbone #(1-3 depending on which sample you used for that transformation)" and then with the amount you plated on that plate (10uL or 500uL). IF DENNIS SITLL HASNT PUT THE SHAKING INCUBATOR BACK: If he is there or there's someone in his office, ask for it back. If not: Initially heat the water bath to 42C. As soon as you put the cells back on ice after heat shocking them at 42C, turn the water bath down to 37C, and throw a good amount of ice in to help it cool quickly. You have 5 mins to get it to 37C or as close to 37C as you can get it. Wedge one of the foam floaters into one of the metal springs so that you can put the tube in the floater and it will shake a bit without risking it going under water. Turn on the shaking function of the water bath to 225rpm and shake for an hour in that instead of the shaking incubator.