MGM100 *in vivo* experiments

Glucose media: Arabinose media:

LB LB

Kanamycin at 50 µg/mL (1L: 1 mL of stock) Kanamycin at 50 µg/mL

Ampicillin at 100 µg/mL (1L: 1 mL of stock) Ampicillin at 100 µg/mL

Chloramphenicol at 100 µg/mL (1L: 1 mL of stock) Chloramphenicol at 100 µg/mL

Glucose at 0.2% w/v (1L: 10 mL of stock) Arabinose at 0.2% w/v

IPTG at 0.1 mM (1L: 0.1 mL of stock)

Note: do not dilute bacteria with water- must use an isotonic solution- LB

Day 1: morning: make arabinose media plates, 1 for each DNA to be transformed

Afternoon: transform DNAs into competent MGM100 cells

1. Thaw DNAs to be tested
2. Place 100 µL aliquots of competent MGM100 cells on ice, let thaw on ice
3. Add ~200 ng of each DNA to cells, flick tube gently to mix (1 µl of plasmid and 1 µl of mHsp10 or GroES)
4. Let tubes incubate on ice for 30 minutes
5. Heat shock at 42°C for 30 seconds
6. Immediately place tubes on ice and incubate for 2 minutes
7. Add 1 mL LB/100X arabinose and incubate tubes at 37°C with shaking for 1 hour
8. Spin tubes at 4000 rpm for 2 minutes to gently pellet cells
9. Remove and discard 800 µL of supernatant (so only ~300 µL of liquid remains), gently resuspend the cell pellet
10. Pipette cell suspension onto labeled arabinose plates, spread with sterile glass beads or sterile glass rod

Day 2: morning: make glucose media plates. Calculate how many plates based on mutant protein combinations and temperatures to be tested.

Afternoon: start overnight cultures of colonies in 2 mL of arabinose media

* 2 ml LB
* 20 µl 100X Arabinose
* 2 µl 1000X
  + Kanamycin
  + Ampicillin
  + Chloramphenicol

Put glucose media plates in 37°C room, upside down, overnight

Day 3: growth experiment

1. Before pipetting any liquid out of any tube, flick gently several times to mix cells (when tube is stationary, cells sink to the bottom)
2. Combine 800 µL of plain LB and 200 µL of overnight culture in a cuvette, mix, read absorbance at 600 nm
3. Calculate the dilutions for each culture to normalize absorbances
   1. (100 µL) (0.6 OD) = (x µL) (diluted OD \* 10)
4. Create serial dilutions for each culture. Gently mix each tube before pipetting
   1. Tube from step 3 is undiluted, 10 ^ zero
   2. To create tube 10-1, combine 90 µL of plain LB and 10 µL of tube zero
   3. To create tube 10-2, combine 90 µL of plain LB and 10 µL of tube -1
   4. Repeat steps to dilute culture to -7 (total of 8 tubes per overnight culture)
5. Label all plates
6. Pipette 10 µL of each serial dilution onto plates, mix each tube before pipetting. When making drops on agar, do not let the pipette tip scrape or puncture the agar.
7. Let all spots be absorbed completely by agar, when surface is dry put plates in sealed Ziploc bags with a moist paper towel
8. Move plates to 37°C or 42°C, incubate overnight upside down

Day 4: scan plates and save results

Scanning: Use 4 inch x 6 inch portrait setting, black and white document, 600 dpi, save as TIFF file