Monday 7/27/09

Plate overnight stock for colony PCR of Pu promoter

- *Media prep*: Sodium Benzoate Plates
  - Autoclave
    - 6 g of agar
    - 100 mL of DI water
  - Directly after autoclaving (agar is concentrated so it will solidify shortly after removing from the autoclave, place ingredients in 37C incubator to help prevent this)
    - 40 mL of 5X M9 Salts
    - 200 uL of 1M $MgSO_4$
    - 58.8 uL of sterile DI water
  - When cooled to 50 C
    - 2 mL sodium benzoate stock
      - Sodium benzoate stock
        - 3 g sodium benzoate
        - 10 mL DI water
    - 20 uL of 1M $CaCl_2$
    - 1mL of 40% glucose solution
    - This will pour about 6-8 plates. Plates may have bubbles if agar starts to cool when in a more concentrated form. If this happens flame the plates.
- *Inoculation*
  - Using 200 ul pipette tip grab a stab and melt frozen stock near the side of the plate
  - Streak out 3 times with inoculating loop
  - Wrap edges with perifilm and place in the 30C incubator

Run gel of the digest

From Jeremy's notes Gene Sequencing Protocol rob for isobutanol evolution

1. Prepare 50 mL of 0.7% agarose gel. Add 0.35 g of agarose to 50 mL of TAE buffer and boil in microwave until all agarose is melted. Stop microwave every 20 to 30 seconds and swirl the solution to help the agarose dissolve. Required about 2 minutes for all of the agarose to dissolve.
   a. 50mL 1X TAE buffer
      i. 10 mL of 50X TAE
      ii. 490 mL of DI water

2. Let agarose gel solution cool to about 55C (hot but not burning to the touch). This will take only a few minutes. Take gel solution to the Gulari lab and add 10 uL of ethidium bromide (10 mg/mL). Swirl solution to mix for at least 30 seconds.
3. Get about a 9 cm gel cast and put barriers at each end (make sure they are snug). Pour gel solution into cast, try not to create bubbles/foam.
4. Use pipette tip to pop any bubbles. If bubbles will not pop, use tip to move the to the sides of the gel.
5. Put comp into gel to create wells. Use the 13x comb. Put the comb near the beginning of the gel, second notch from the rubber stop. Leave gel on bench 20-25 minutes to solidify.
6. While gel is solidifying prepare samples
   a. Mix digest with Blue juice lading dye in 5:1 proportion in PCR tubes. Prepare 6 uL total volume
      i. Smaller sample volume avoid smearing
      ii. 4.8 uL of DNA and 1.2 uL loading dye
   b. For 1 kb ladder use concentrated stock in -20C fridge, enzyme box 2
      i. 0.5 uL of concentrated ladder (yellow PCR tubes labeled L)
      ii. 3.5 uL of DI water
      iii. 1 uL of blue juice
7. After gel is solidified, remove comb and casting blocks. Put gel in holder, orient so wells are by black electrode (DNA migrates towards +, the positive electrode)
8. Put fresh TAE buffer in the electrophoresis tank
   a. After one or two runs, the buffer becomes depleted!
9. Load 5 uL of PCR products and ladder
10. Set to 85 V run 1 h to 1 h and 30 minutes
11. Take picture by lifting cover and placing on UV light
    a. Go to mode, M1 for best picture quality
       i. Night mode
       ii. Macro
       iii. Slow exposure

Plating Pu luciferase part sent from registry

1. Stab agar from tube with inoculating loop
   a. Duplicate parts sent so do once for each culture
2. Streak on plate with appropriate antibiotic resistance (AMP in this case)
3. Repeat streaking two more time
4. Incubate at 37C

Results

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Gel of RBS and Bacteriophage lysis cassette cut with EcoRI and XbaI

Samples

Lane 1 (Far right): invitrogen 1 kb plus ladder

Lane 2: RBS colony 1

Lane 3: RBS colony 2

Lane 4: RBS colony 3

Lane 5: Bacteriophage lysis colony 1

Lane 6: Bacteriophage lysis colony 2

Lane 7: Bacteriophage lysis colony 3

Expected fragment lengths

- RBS
    - Two expected fragments
        - 58 bp promoter region
        - 2091 bp plasmid backbone with RBS
            - Plasmid backbone 2079 bp
            - RBS 12 bp
- Bacteriophage lysis
    - One expected fragment
        - 4473 bp Plasmid backbone with lysis cassette

            - Plasmid backbone 3189 bp
            - Lysis cassette 1284 bp
                - Part 1257 bp
                - Primers
                    - Prefix 16 extra bp
                    - Suffix 11 extra bp
    - Brown lysis part is not consistant, can check sequence in registry, the part is really 267 bp of terminator + terminator +pBAD promoter without araC
        - Our gel matches this prediction with a total part length of 3456