18/6/2013

MidiPrep and Purification of pQE30:αSNAP

- Number “70” Cells @ 100mL
- 2 Midipreps, 50mL starting culture each
- Final: 2 samples of purified plasmid DNA @ 1.0mL
    - Will run samples on gel after restriction to determine viability and yield of midiprep
    - NanoDrop:
        - Sample One: 66.8 ng/µL
        - Sample Two: 99.1 ng/µL
    - Restriction was unsuccessful; Invitrogen enzymes not compatible in same buffer

Preparing the constructions of pLac-RBS-mCherry and pLac-RBS-mRFP1

2/7/2013
First Transformation

- Used XLI Blue Cells, cultured for aprox. 2 hrs.
- Gathered Biobricks:
    1. RBS-mCherry-t-t (BBa_J06702) [CamR]
    2. pLac-RBS-mRFP1 (BBa_K741002) [CamR]
    3. pLac-RBS-GFP-t-t (BBa_I715039) [CamR]
    4. pLac-RBS-mRFP-term (BBa_J04450) [KanR]
    5. pLac (BBa_R0010) [AmpR]
    6. pQE30:αSNAP (+ Control) [AmpR]
    7. dH_2O (- Control)
- Completed Transformation, completely unsuccessful
    - I think it may be due to the cell cultures; extremely sensitive at certain period during exponential phase, will take extra precautions during this phase

4/7/2013

- Re-performed Transformation with same protocols, only taking into account the exponential phase issue.
    - Successful on all plates

5/7/2013-8/7/2013

- Cultured colonies and miniprep the biobricks for standard assembly
- Standard Assembly of three biobricks:
    - pLac + RBS-mCherry-t-t + Backbone (pLac-RBS-GFP-t-t)

9/7/2013

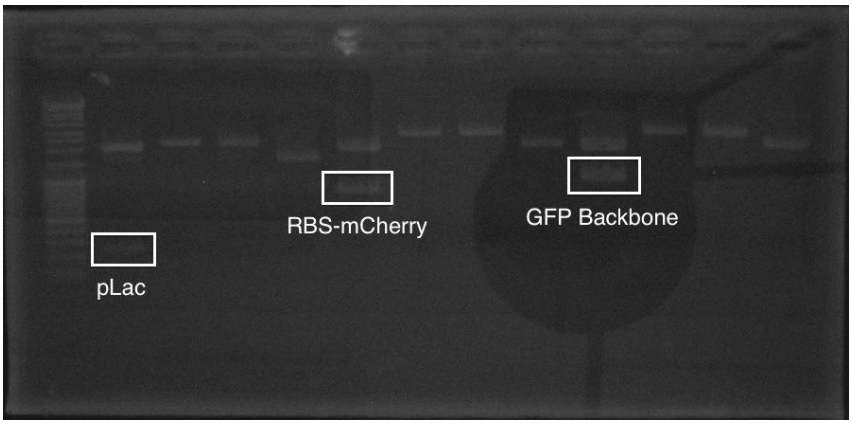
- Restriction/Gel Electrophoresis
    - Biobricks were restricted in quadruplicate (for control) and placed into wells of agarose gel; Failure, must re-perform the restriction
- Cultured colonies for standard assembly
    - pLac, RBS-mCherry, pLac-RBS-GFP, and pLac-RBS-mRFP

10/7/2013

- Miniprep the biobricks for standard assembly
    - pLac [238.2 ng/µL]
    - RBS-mCherry [180.8 ng/µL]
    - pLac-RBS-GFP [183 ng/µL]

11/7/2013

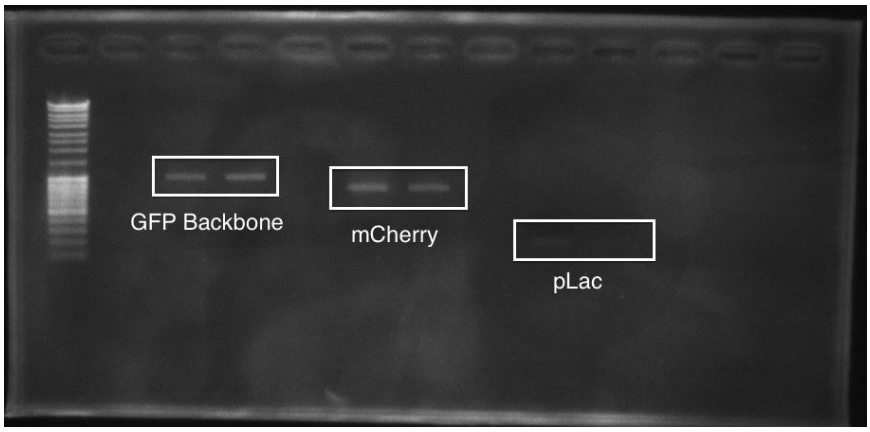
- Performed restriction using standard assembly followed by Gel Electrophoresis (1.2% Agarose @100V for approx. 20 min.)



- Gel Extraction and Purification:
    - Final Concentrations were negligible on the nanodrop

12/7/2013

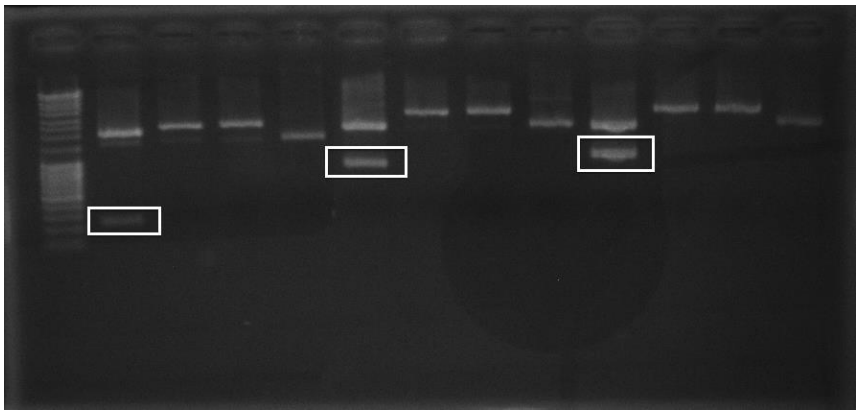
- Ran products on a gel once more and all DNA samples were present, only pLac was slightly unclear
- Will run side-by-side restrictions to increase yields



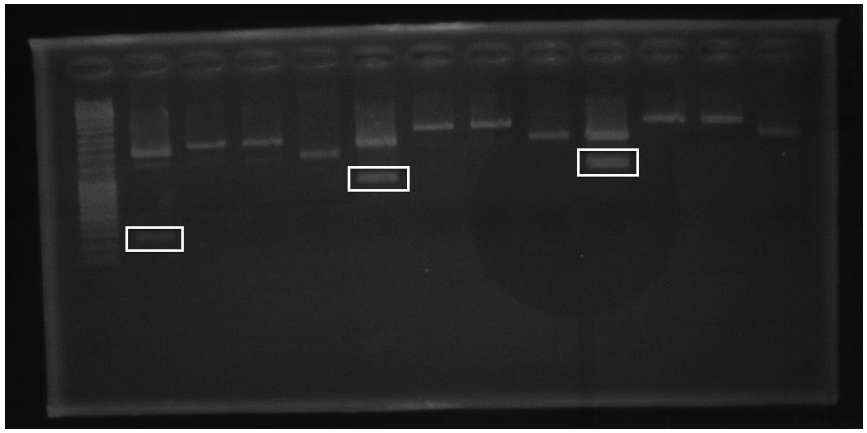
15/7/2013

- Restriction in Standard Assembly for three biobricks (third attempt)
  - This restriction was performed in duplicate:
    - 2 separate restrictions (A&B), 2 Gels (A&B)
    - The gel samples were extracted and placed together in the centrifuge tube for purification
  - Electrophoresis:

A



B



15/7/2013 cont.

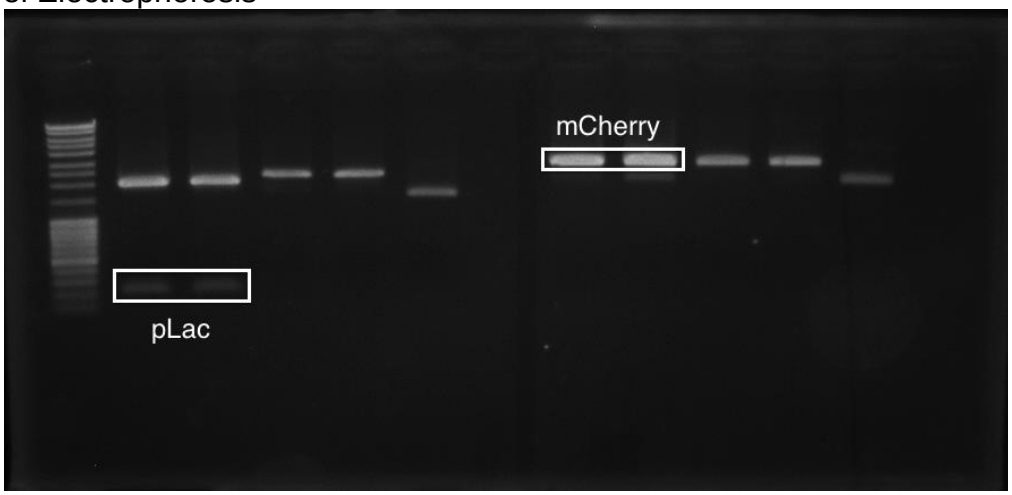
- Extraction and Purification (Qiagen) of the restriction products
    - pLac [5.4 ng/μL]
    - RBS-mCherry [6.5 ng/μL]
    - pLac-RBS-GFP [8.3 ng/μL]

17/7/2013-18/7/2013

- Ligation (Promega) at 1:3:3 Ratio, Room temperature for 3 hours.
    - Plates overnight did not grow, re-diluted cells in order to culture backup cells with CAM
    - Concentrated the remaining JM109 to plate once more
- Ligation was unsuccessful, will restart from restriction. Must find alternate route.

23/7/2013

- Restriction in “Standard Assembly” of TWO Biobricks
    - In this constructions, pLac will be inserted directly in front of RBS-mCherry
    - Performed in duplicate to concentrate final product
- Gel Electrophoresis



- Gel Extraction and Purification
    - pLac [2.7 ng/μL]
    - RBS-mCherry [20.2 ng/μL]

24/7/2013

- Ligation (Promega) at 1:3 Ratio, Room temperature for 3 hours
- Transformation using JM109 Cells
    - 4 Setups:
        - 1:3 Ratio/20 μL
        - 1:0 Ratio/20 μL
        - 1:3 Ratio/10 μL
        - pQE30:αSNAP (+ Control)

29/7/2013

- Observations:
    - After transforming all setups on 24/7, the only plate with colonies was pQE30:αSNAP (+ Control). For the previous transformation, I followed the advice of one of our advisors, who mentioned that keeping the cells at room temp. overnight and plating the following day could help if no colonies grew. On 25/7 (the day following the transformation), I replated the 1:3/20 and 1:3/10 ratios of mCherry and left the plates at 37°C over the weekend. After checking the plates of 29/7 (Monday), I found that both ratios had grown and formed red colonies! Success!
    - Number of Colonies:
        - 1:3/20 = 25 colonies
        - 1:3/10 = 37 colonies
- I will now make glycerol stocks of JM109 mCherry and begin liuid cultures for miniprep for M15 transformations

30/7/2013

- Miniprep
    - 2 minipreps were prepared (one for each ligation ratio plate)
    - Will use to transform pLac-RBS-mCherry into M15 cells
    - Final Concentrations:
        - 1:3/20 [103.1 ng/μL]
        - 1:3/10 [90.7 ng/μL]

31/7/2013

- M15 TSS Transformation
    - Transformation was successful on all 3 plates
        - 1:3/20 = 275 colonies
        - 1:3/10 = 202 colonies
        - pQE30:αSNAP = 29 colonies
    - Started a liquid culture of M15 cells containing mCherry
        - Will make glycerol stocks