PHA Synthesis Lab Notebook:

Week 1 – June 16

1. Styene team has already assembled and is making plastic
2. We researched biodegradable plastics using the chart that Forrest made – previous teams have made P(3HB) so next week we will try to see how we could improve.

Week 2 – June 23

1. Learned about Tokyo Tech team and Imperial 2013 team, identified opportunity of using panK gene
2. Kosuke helped us design gene with proper prefix and suffixes

6/30/2015

1. Did bleach extraction with the Imperial Team Hybrid Promoter
    a. Bleach Extraction of Imperial Promoter shows that there might be more plastic produced.
2. Looked under microscope but result is inconclusive

07.01.2015

1. PCR of PanK was successful, molecular cloning into pSB1C3 Pha-BAC & digest
2. Growth of hyb PHA for Forrest to look under microscope during Bleach extraction protocol w/ negative ctrl

7/1/2015

1. Do digestion and then ligation to plasmid
2. Transformation and compare growth? - How
3. Do extraction procedure today on the plasmid
4. Think about the cell free system

7/7/2015

1. Did ligation and transformation of Pank to the Imperial Hybrid Promoter
2. Charles finished extraction procedure of the Imperial vs the negative control (RFP)
    a. The Imperial produced plastic while the negative control does not
3. Tested Nile Red - added Nile REd and incubated for 30 minutes - results are still inconclusive

7/8/2015

1. Checked transformation plate and there is colonies
2. Did colony PCR on Pank/Hybrid
3. Needed to do overnight culture of Pank/Hybrid and Miniprep
4. Nile Red Verification again

Future Week Plan

1. Quantitatively determine how much P3HB (Thai and Erica)
2. How to use LC/MS - purification protocol - next week (Thai and Erica)
3. Nile Red + flow cytometer - Pank Test to see if actually increases production
4. Conversion of the powder into plastic (Forrest)
    a. Look into PHBV - (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3147381/)
5. Figure out gene and order them for secretion or lysis mechanism (Thai, Charles, and Erica)
6. 3D Printing - later
7. Enable E coli to grow in sucrose (PowerCell) - http://aem.asm.org/content/60/4/1198.full.pdf+html

7/16/2015

1. Send Pank2, Pank 4, Imperial 1, Imperial 2 for sequencing - last time result gives terrible results - possibly because of having two primers
2. Need to talk to downstair team tomorrow
3. Did autolysis biobrick - will use BBa_K317039 (order) and BBa_K0820930
    a. Talked to Tyler about possible model for autolysis sytem
4. Can do sequencing after colony PCR and PCR clean up
5. Learn about Western Blot Method used by Dan's group today

. Acetic Acid has pungeant smell - used for wash

6. Learned about fluorometer and OD measure

. Visible Light -> measures OD at 600

a. Fluorometer -? 200 ul per well in 96. Go to setting -> endpoint -> Fluorescent (RFU) -> Wavelength -> Excitation/ Emission

b. Need to do this tomorrow

7. Learned about CRISPR Cas method

. Use Addgene Cas 9 Protocol

8. Overnight Culture 3 ml for 6 Spore - tomorrow put in starving media

7/17/2015

1. Sequence Result successful - need to do reverse sequencing and internal sequencing
2. Talked to lab downstair - very helpful with the project
3. Made overnight culture successful

7/20/2015

1. Need to buy P(3HB) from Sigma aldrichhttp://www.sigmaaldrich.com/catalog/product/aldrich/363502?lang=en&region=US
2. Propionate Sequence: http://www.ebi.ac.uk/ena/data/view/CAB77207 mismatch one protein with the yale pct gene http://onlinelibrary.wiley.com/doi/10.1046/j.0014-2956.2001.02659.x/epdf Will there be any effect from this site directed mutagenesis?
3. Need to buy sodium hypochlorite
4. Synthesize Orz gene for P(4HB)
5. Sequencing Primers for Pank internal
6. Send an email asking about Pct gene
7. Do Fluorometer and flow cytometry experiment toward the mutant
8. Transform waterproof and
9. Need to buy P(3HB), sodium hypochlorite, propionic acid
10. Sang Yup Lee 2009 Paper explains mutation(PctCp mutant having V193A mutation, and four silent nucleotide mutations of T78C , T669C, A1125G, T1158C) (Table I). PhaC1310 Ps6-19, which resulted in both relatively high polymer content and Mw among various mutants of PhaC1 Ps6-19, and Pct540 Cp, which led to the increase of both polymer content and lactate mole fraction in the copolymer among various mutants of PctCp, were selected as the enzymes to be introduced into E. coli for the production of PLA polymers

7/21/2015

1. Purchased PLA, P(3HB-co-3HV), sodium hypochlorite, 4HB precursor, sodium propionate,
2. Discuss cellulose binding domain
3. Extract plastic - dry weight of plastic for Pank is much more
4. Overnight Culture for lysis gene and Imperial and pank + imperial gene
5. Transformed lysis gene onto plate and cellulose binding domain gene

7/23/2015

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0063442

Talks about comparing Pelb signal peptide to MalE

1. Pel B Sequence: ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCC

9/14/2015

1. Check sequencing for autolysis, birA, pank, and CIPA3
2. This week tasK:

a. Finish Wiki
b. Do lysis testing
c. Do autolysis testing
d. Extract BirA and Cipa3
e. Sequence BirA and Cipa3

8/5/2015

1. miniprep T7 + PctCP DNA
2. nanodrop, then digest with biobricks enzyms
3. perform PCR purification – use all 40uL into small tubes, follow Qiagen protocol
4. nanodrop backbone and vector, need 3X as much vector for ligation
5. Ran vector with Danny's gel – expect 2kb and 1.5kb
6. Ligate and transform
7. Plate T7+Pct+Imp+panK
8. Do overnight cultures of T7+Pct+lysis system *2
9. Note: TB/chlor is old, chlor degrades in light D:

8/6/15

1. colony PCR to find out if any cultures have T7+pct, melA, lysis
2. tried modeling how fluorescence should increase with cell size according to flow – talked to Dans

8/7/15

1. flow cytometry w p3hb
2. 1:5 dilution in PBS
3. spin down, resuspend in PBS, dilute 1:5, add nile red, rock in foil, flow immediately
4. plan for rest of week of panK characterization
5. larte n=6 experiment to start for flow and extraction

8/8/2015

1. transform luxL generator for lysis
2. transform pTet for lysis
3. can be induced with L arabinose
4. replate melA on amp plates bc of orange top
5. can all be transformed into DH5a except pctcp – go in lysY t7 express

8/9/2015

1. cal academy – meet with canopy meg
2. presentation preparations

8/10/2015

1. make large culture of TB from protocol online
2. ligate t7 to Pct again – didn't work last time.
3. Overnight cultures of ptet and luxl

4. Colony PCR of birA, cipA, T7+pct, and panK (starting cellulose binding)

8/14/2015

1. mini prep all plastics
2. flow
3. check sequencing

8/17/2015

1. analyze flow data
2. freeze dry plastic cultures
3. check sequencing
4. fluorescence/OD600 testing
5. fluor – dilute 1:10 in PBS, 200uL cells in each well

8/18/15

1. large cultures didn't all dry out because tubes were sealed
2. used sodium hypochlorite for future extractions
3. must break up plastic beforehand with pipette (use metal next time)
4. plastic extraction: centrifute 5 min at 12000g, add ethanol and vortex, centrifuge, discard super, add water, allow to evaporate overnight

8/20/2015

1. minipreps for interlab
2. run ligation, transformation for interlab

8/22/2015

1. digest, enzymatic cleanup, ligation, transformation for all interlab
2. plated for Kirsten (see interlab notebook)

8/28/15

1. nile red staining for flow and fluorescence
2. plastic extraction for mass tomorrow
3. dilute 1:10 for plate readers, also for flow

8/31/2015

1. plastic extraction complete with new NaOCl
2. they all exploded in incubator, must use bigger vials next week.

9/2/15

1. plan for second cal academy visit!
2. Miniprep lysis 1&2 and also stuff for thai and Charles
3. Lysis experiment – add .150g arabinose and measure OD600 over course of several hours.
4. Pbad promoter from Berkeley construct worked at 1000uM and best to add mid-log
5. Use LB chlor for blank

9/4/15

1. panK fluorescence testing – follow normal staining protocol
2. extraction – lyophilize over weekend

9/9/15

1. 15 plastic extractions – make sure they do not explode
2. keep containers open as long as possible
3. let dry overnight at 40C, then 60C if not dried

9/11/15

1. lysis testing in OD600 – questionable results

9/16/16

1. lysis testing again – still questionable but more promising.