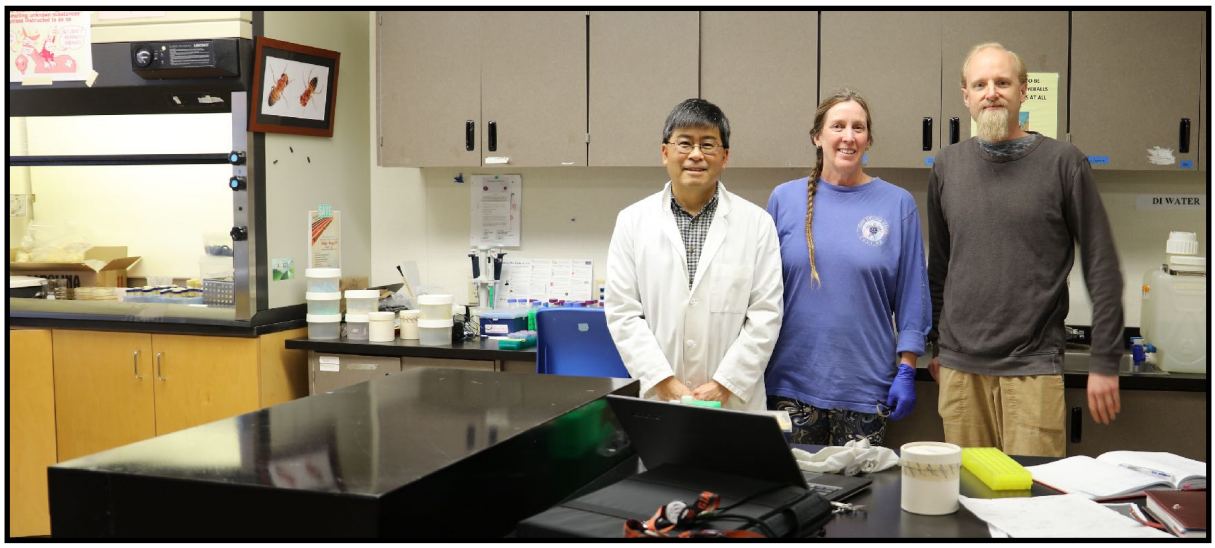
Project:

Rendering a Biosynthetic Carotenoid Pathway

ARC/BAC (American River College/ Biology & Art Collaboration)
Art/Science Research

Purpose: To use biotechnological tools and methods as a means to artistically "render" color through through construction of a synthetic molecular pathway.



ARC/BAC (American River College Biotechnology and Art Cooperative) Members: Dr. Ken Kubo, Artist Carolyn Angleton, Dr. Adam Telleen

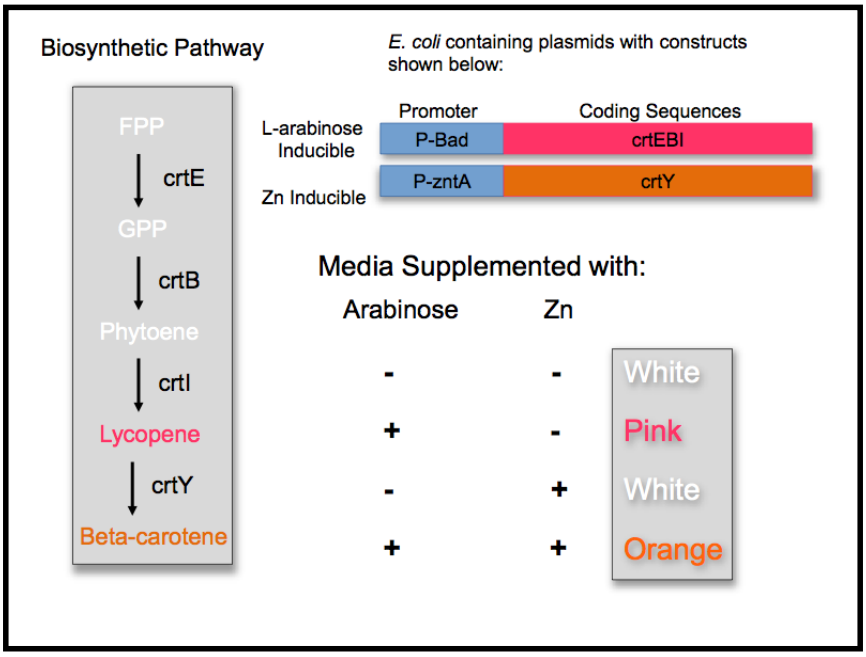


Diagram showing the Biosynthetic Carotenoid Pathway and plasmid constructs

Protocol:

1. Design genetic constructs. Identify what genetic sequence to use, what it will look like, what primers to use.

2. Identify source DNA-we used genomic DNA from *Pantoea agglomerans,* a yellow pigmented rod-shaped gram negative bacteria. This particular bacteria was chosen for three reasons: 1) A partial genomic analysis has been done and provides us with a known genomic sequence, 2) It produces yellow pigmentation, 3) it contains the genes crtE, crtB, crtI, crtY -which are activated in the carotenoid pathway.

3. Generate gene inserts of the carotenoid bio synthetic genes (crtE,crtB, crtI, crtY) by Phusion PCR (polymerase chain reaction).

4. Create vector DNA by Phusion PCR using pGLO and pUC plasmids. These will provide the vector backbones-each with a different selective marker and origins of replication (ORI).

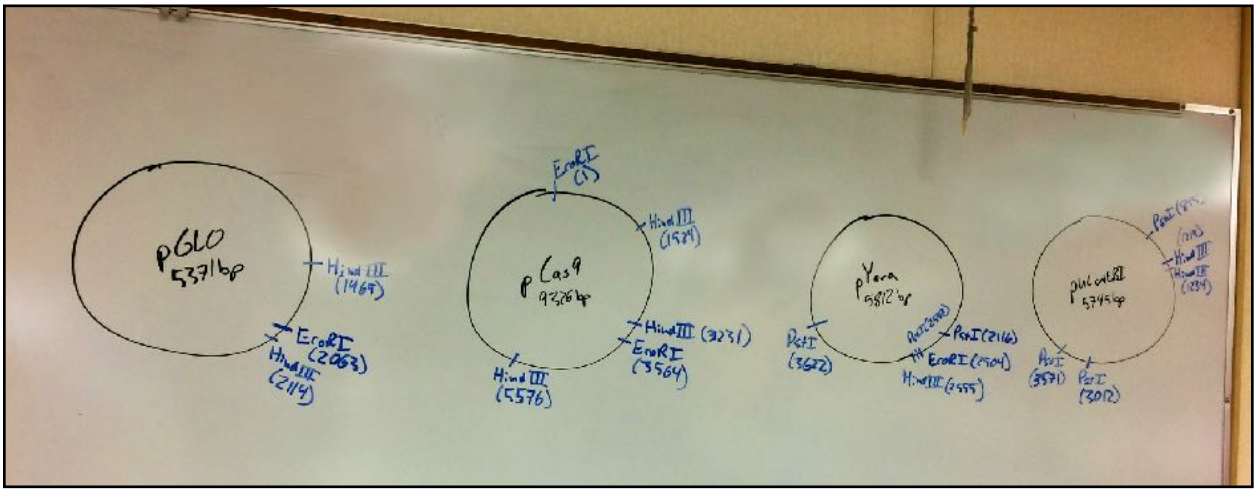
5. Combine inserts with vector DNA backbone which contain inducible promotors (crtEBI induced by IPYG, crtY induced by arabinose).

6. Use Gibson Assembly to construct the recombinant plasmids that contain the genes. Make 2 different constructs: 1) crtY in pGlo, 2) crtEBI in pUC

7. Shuttle crtY gene construct along with regulatory sequences from pGLO into pCas9 vector backbone.

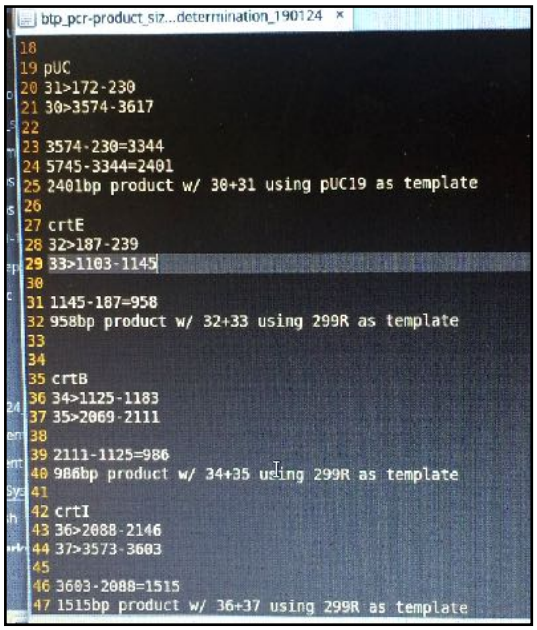
8. Introduce synthetic constructs into *E. coli* and assess pigment coloration.

9. Test under different conditions:
   -If CRT EBI is working, it should change colorless bacteria into pink colored bacteria.
   -with lycopene present from first construct (EBI), If CRT Y is working, the pink should transform into orange.

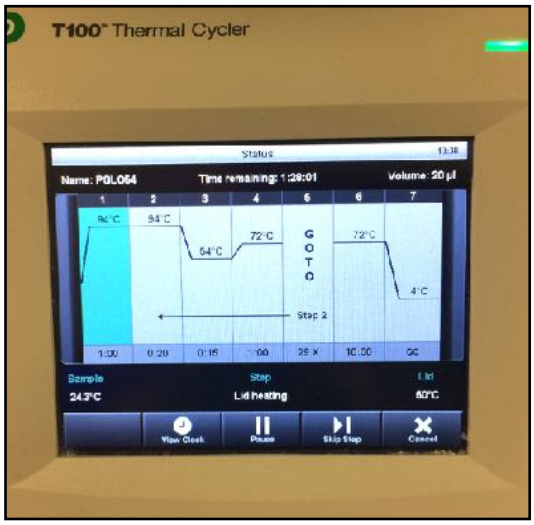


Experimental Design diagrams, restriction site plasmid map drawings

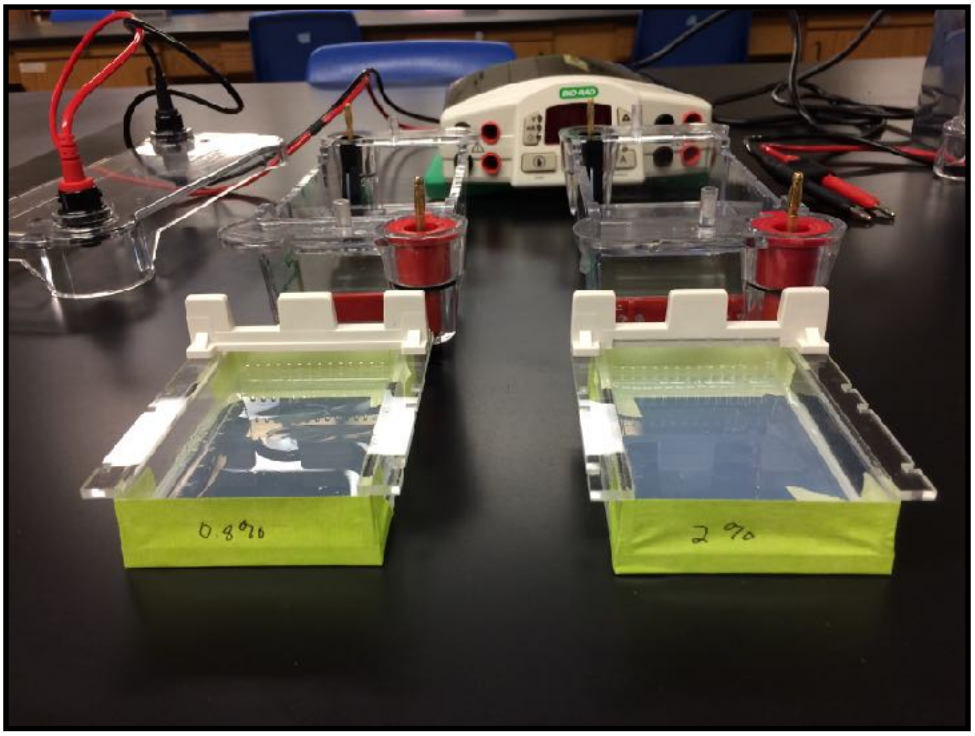
Order primers and plasmids



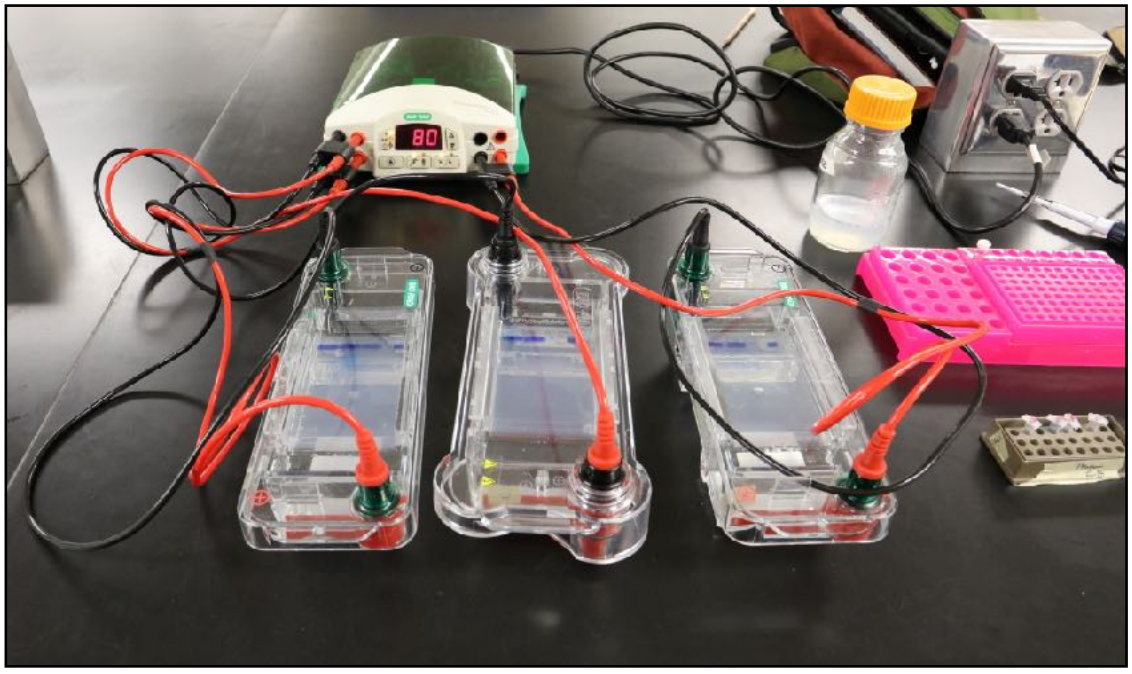
PCR product size determination; computer research



Phusion PCR process: Microtubes containing samples of the gene insets crtE and crtY in thermocylcler, a programable machine that cyclically raises and lowers heat temperatures to allow for the denaturation, annealing, and extension of DNA products in order to amplify specific segments of DNA.



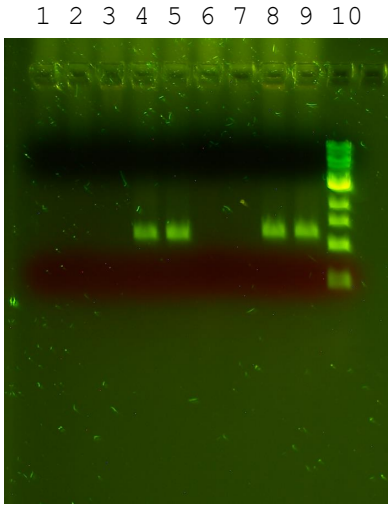
Agarose gels: different concentrations of agar allow us to control speed of DNA movement through gel matrix



Gel electrophoresis units running simultaneously to quantify multiple batches of DNA

	DNA sample	Primers
1	3	40/41 (crtY)
2	4	40/41 (crtY)
3	12	40/41 (crtY)
4	20*	40/41 (crtY)
5	P agglomerans*	40/41 (crtY)
6	3	40/R2107 (crtY-pGLO)
7	4	40/R2107 (crtY-pGLO)
8	12*	40/R2107 (crtY-pGLO)
9	20*	40/R2107 (crtY-pGLO)
10	kB ladder	

*PCR product



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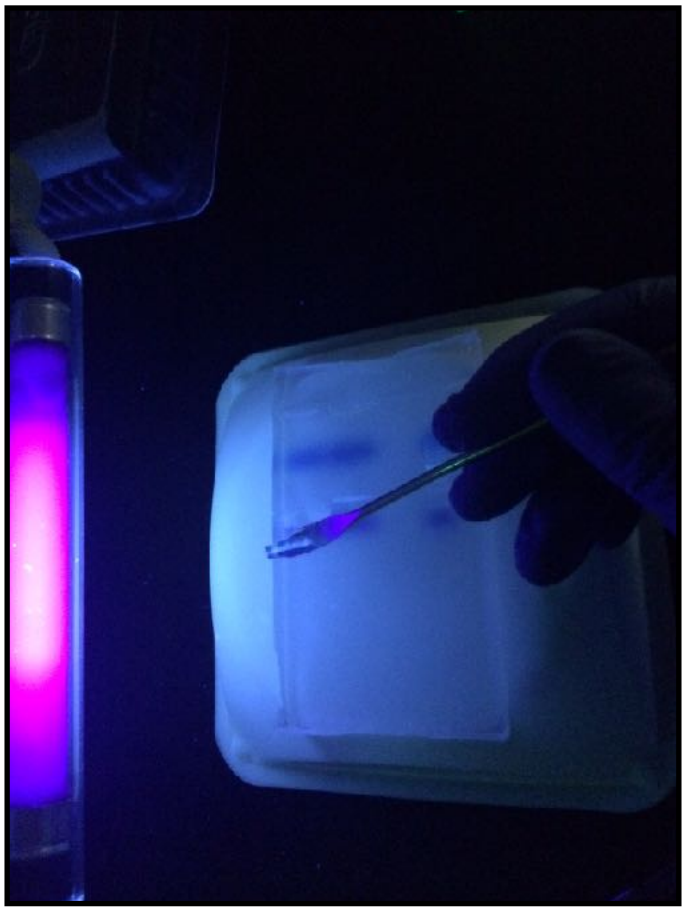
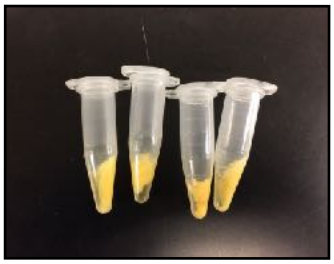
Results of gel electrophoresis, Lanes 4, 8, 9, show positive results. Lane 10 is the ladder. After the electrophoresis is done running, the gel is illuminated w UV light in order to identify if protein band is present and if so, its base pair size.

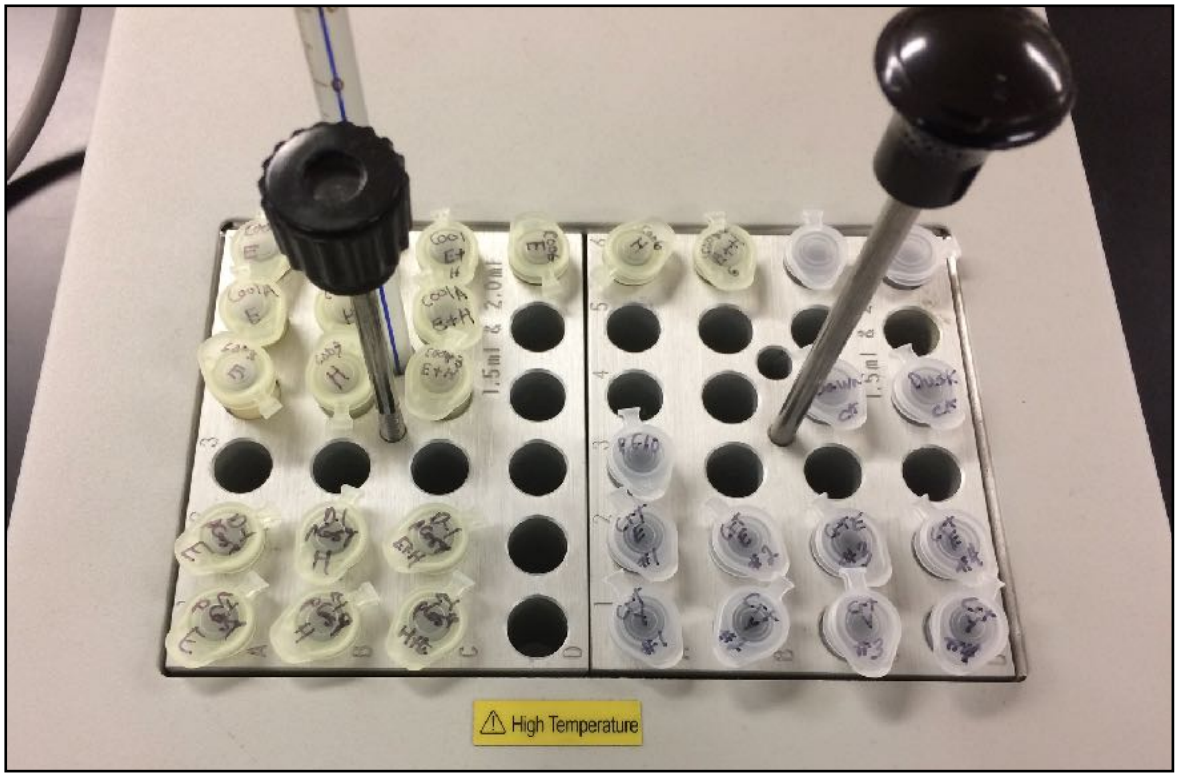


Gel Extraction; use scalpel to cut out band containing gene of interest

Zymoclean Gel DNA Recovery Kit used to process and cleanup the DNA

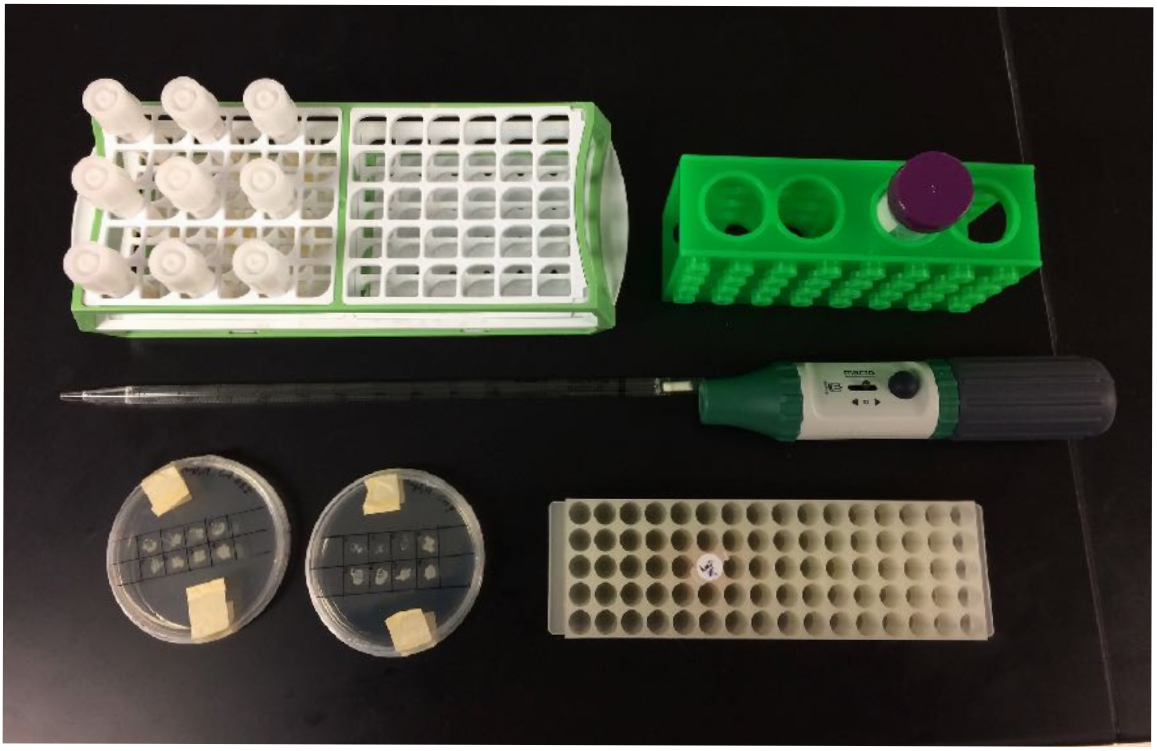


Purified DNA samples

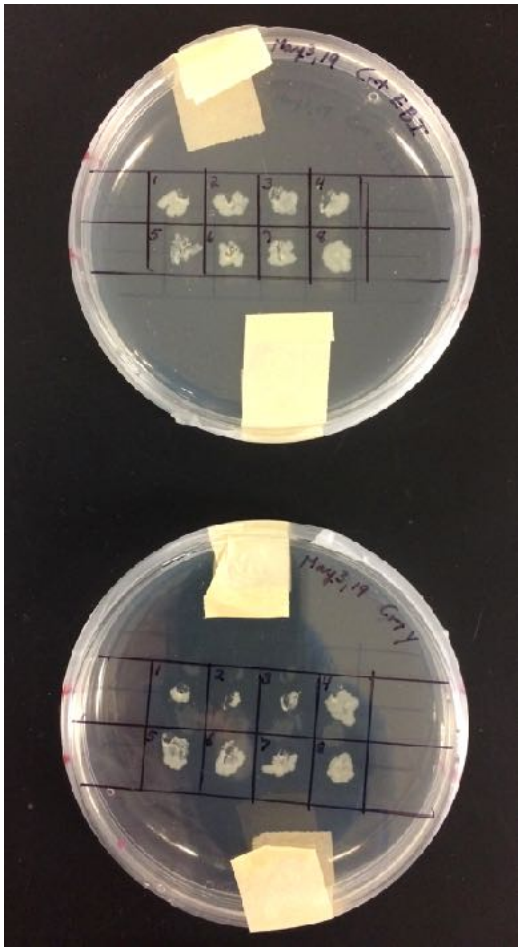


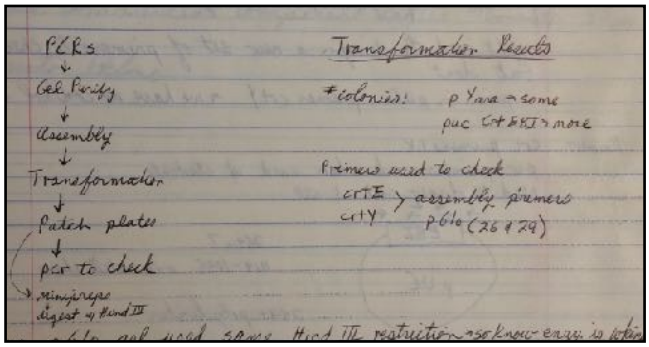
Heat shock Transformation: A sudden increase in temperature creates pores in the membrane of competent and allows for plasmid DNA to enter the bacterial cell

Pouring LB Agar plates to be used to inoculate and grow *E. coli* containing the gene insert

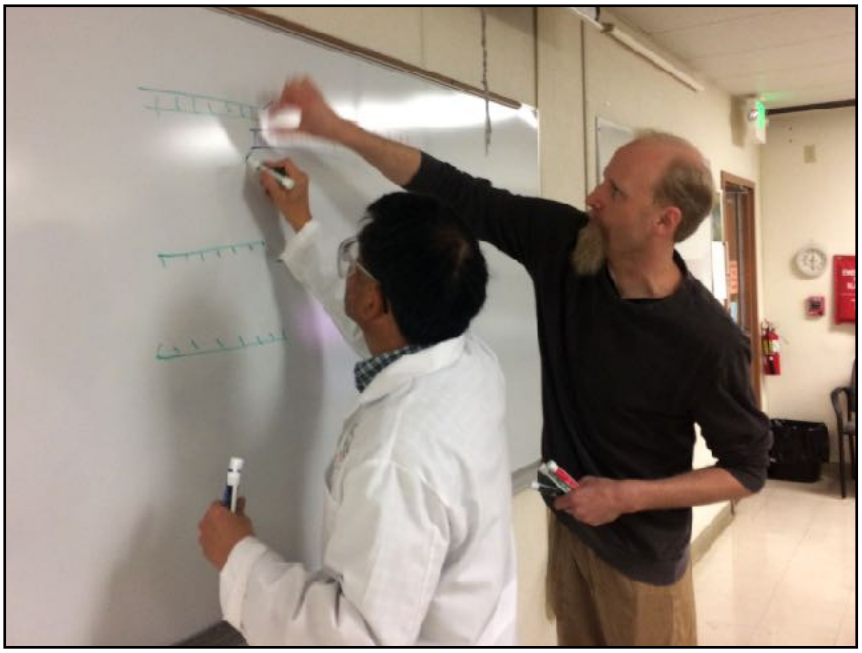


Broth cultures and patch plates -plating individual colonies to see if they contain plasmid





crtEBI and crtY Streak plates



New results, new problems- back to the drawing board