Lethbridge HS iGEM

Laboratory Notebook 2017

20170330 transformation from Kit Plate (2015)

Names: Kyle, Nimaya, Candace, Stefanie, Ross, Kyle, Haroon, Bradley

Purpose: Introduce plasmids into cell for later purification

Plasmid name(s): BBaK516132, BBaK608008, J23100, J23101, J23102

Plasmid description:

- Size: (pSB1C3_BBa_K516132- 2975 bp) (pSb1C3_BBa _K608008- 2852 bp) (pSB1C3_J23100- 2105 bp) (pSB1C3_J23101- 2105 bp) (pSB1C3_J23102- 2105 bp)
- Resistance: Cam
- Other (inserts, components, origin, notes):
  BBa K516132 con-promoter, mRFP, RBSB0032

  BBa k608008 con-strong-promoter, medium RBS, GFP

  J23100,101,102 Anderson Promoter Collection

Cell Strain: DH5a

Positive Control: pUC19

Negative Control: Water

Results:

Number of colonies on:

- Experimental: 0
- Positive control: >300
- Negative control: 0

Conclusion:

The lab process was correct because the positive control produced growth. Nothing was contaminated during the process of both the negative and positive controls.

On the other plates, possible error could have been caused by the age of the DNA because it was from 2015.

Additional Notes:

-Plated all kit plate plasmids on LB containing Cam
-Plated the pUC19 control plasmid transformation on Cam and Amp plates
-Plated water negative control on Cam plates

20170331 transformation

Names: Autumn, Kelly

Purpose: Introducing plasmid to cells to replicate Anderson promoters.

Plasmid name(s): BBa_K823007_pSB1C3, BBa_K823008_pSB1C3

Plasmid description:

- Size: 2105bp
- Resistance: cam
- Other (inserts, components, origin, notes):
  BBa: Anderson promoter J23103

  BBa: Anderson promoter J23106___

Cell Strain: DH5a

Positive Control: pUC19

Negative Control: water d2H20

Results:

Number of colonies on:

- Experimental: 0
- Positive control: >300
- Negative control: 0

Conclusion: The samples from 2015 may be too old to use as only the positive control yielded results, not the experimental. Nothing was contaminated as shown in the negative control with no growth.

20170403 Transformation

Names: Kyle, Autumn, Erin

Purpose: Introducing plasmids into cells to produce two gfp as well as a promoter and binding site.

Plasmid name(s): 2016 igem kit plate, 3O, 10A and 13C

Plasmid description:

- Size: 2615, 2989, 2125
- Resistance: cam
- Other (inserts, components, origin, notes):
  3O contains a strong promoter and rbs. 13C contains con promoter and gfp generator. 10A contains J23101 RBS and gfpi with no terminator.

Cell Strain: DH5a

Positive Control: pUC19

Negative Control: none

Results:

Number of colonies on:

- Experimental:____0_____
- Positive control:____>300____
- Negative control:___0_____

Conclusion: DNA in the Kit plates may be damaged as they are not transforming. The transformation process is working because the positive control is growing well.

To try next:

- PCR amplify parts from kit plate and assemble from there
- Find parts from previous years to transform freshly

20170407 Transformation of plasmid DNA

Names: Kelly, Autumn, Erin, Sydnee

Purpose: Transforming previous miniprep DNA into cells to test if it works better than the kit plate DNA.

Plasmid name(s):
E1010_pSB2K3 - mRFP coding sequence, kan resistant
J04450_pSB3C5 - RFP coding device, LacI inducible promoter, cam resistant

Plasmid description:

- Size: 5131bp E1010, J04450 not determined
- Resistance: E1010 kan, J04450 cam
- Other (inserts, components, notes):
  2012 miniprep DNA

Cell strain: DH5α

Positive control: pUC19

Negative control: water

Added 25µL of IPTG to the media in the J04450 transformation before plating. This part in under the control of an inducible lac promoter that should be activated in the presence of IPTG. hopefully, the cells in the transformation will turn red because the expression of RFP will be turned on

Results:

Number of colonies on:

- E1010_pSB2K3: 0
- J04450_pSB3C5: 0
- Positive control: 0
- Negative control: >300

Conclusion:

The negative control was the only plate to show growth, and the positive control, E1010 and J00450 transformations did not grow. There are a couple possible explanations for these results. Either the water that was used in the negative control was contaminated with DNA, or we mixed up plating the positive and negative control transformations on their respective plates. Maybe early addition of ITPG to the J04450 culture stressed the cells too much and killed them.

We will be repeating this transformation to rule out some of these possibilities.

20170409 Repeat Transformation of E1010 and J04450

Purpose: perform the transformation for E1010 and J04450 again to eliminatete the possible reasons for the results from the previous day. Will also transform J23114_pSB1A3 and J23113_pSB1A3

Plasmid names:

- E1010_pSB2K3 - mRFP coding sequence, kan resistant
- J04450_pSB3C5 - RFP coding device, LacI inducible promoter, cam resistant
- J23114_pSB1A3 – amp resistant
- J23113_pSB1A3 – amp resistant

Added 25µL of IPTG to the J04450 transformation plate.

Cell strain: DH5α

Positive control: pUC19

Negative control: water

Results:

- E1010_pSB2K3: 0
- J23114_pSB1A3: >300
- J23113_pSB1A3: >300
- J04450_pSB3C5 +IPTG: 7 red colonies
- J04450_pSB3C5 -IPTG: 6 pink colonies
- Positive control: >300
- Negative control: 0

Conclusion:

The negative control did not produce colonies as expected, and the positive control grew very well. It is probable that these two samples were mixed up during the last transformation, and we can be certain that we do not have a contamination now. All other transformations except for E1010 were successful. The J04450 transformation plated without IPTG were more white than their +IPTG counterparts. It appeared that the center of the colony was red, while the edges were white. This could be due to leaky expression of the RFP.

20170411 miniprep of J04450_pSB3C5

Miniprep of Plasmid DNA

Names: Nimaya, Haroon, Erin

Purpose: To isolate and purify DNA from bacteria of J04450 pSB 3C5

Plasmid name(s): _J04450 pSB 3C5

Glycerol Stock Location: na

Plasmid description:

- Size: to be determined
- Resistance: cam
- Other (inserts, components, notes): it is a completeRFP expression set

Elution volume: 30 µL

Elution Solution: €TE Buffer

Plasmid	Concentration
-1 J04450 pSB3C5	158.2 ng/µL
-2	178.4 ng/µL

20170419 Transformation

Names: Halla, Nimaya, Erin

Purpose: Introduce plasmids into cells for later purification

Plasmid name(s): J23113 (in pSB1A3), J23114 (in pSB1A3), TAT E1010 (in psB1C3), TAT E1010 (in psB2K3), TAT E1010 (in pGEM (amp))

Plasmid description:

- Resistance: Amp (pGEM, PSB1A3), Cam (psB1C3), Kan (psB2K3)
- Other (inserts, components, origin, notes):
  J23113, J23114 = promoter parts
  TAT E1010 = signal sequence on RFP

Cell Strain: DH5α

Positive Control: pUC19 DNA

Negative Control: D_2H_2O

Results:

Number of colonies on:

- Experimental:

(J23113, J23119, pGEM) >300
(pSB1C3)(pSB2K3) = 1

- Positive control: >300
- Negative control: >300

Conclusion: Negative control grew, may have contamination. Cannot trust results on other transformation plates.

Additional Notes:

*DNA stocks are different than those used before, as the previous ones were not growing

20170509 Miniprep of plasmid DNA

Names: Kelly, Nimaya, Autumn, Erin, Sydnee

Purpose: miniprep DNA for later use.

Plasmid name(s):

J23108 J06702_pSB1C3

E1010_pSB2K3

J23100 J06702_pSB1C3

Glycerol Stock Location: Wieden lab -80 freezer

Plasmid description:

- Resistance: pSB1C3 (cam), pSB2K3 (kan)
- Other (inserts, components, notes):
  Inserts: E1010 → signal sequence in RFP

  J23108, J23100, J06702 → promoter parts

Elution volume: 30 µL

Elution Solution: Water

Plasmid	Concentration
J23108 J06702_pSB1C3	318.5 ng/ µL
E1010_pSB2K3	89.19 ng/ µL
J23100 J06702_pSB11C3	293.6 ng/ µL

Additional Notes:

J23100 J06702_pSB1C3 was less concentrated because less of the cells were used.

20170510 Restriction digestion

Names: Nimaya, Autumn and Allison

Purpose: to cut approx. 500ng of plasmid DNA with two different restriction enzymes. This should provide enough cut DNA for both ligation and agarose gel electrophoresis.

Tube 1:

- Plasmid: J23100 J06702_pSB1C3
- Enzymes used: EcoRI and PstI
- Other:

Tube 2:

- Plasmid: E1010_pSB2K3
- Enzymes used: EcoRI and SpeI
- Other:

Tube 3:

- Plasmid: J23108 J06702_pSB1C3
- Enzymes used: SpeI and PstI
- Other:

Agarose gel conditions:

- Percentage: _____1%_____
- Voltage: _____135V_____
- Run time:_____25 min_____

Lane	Contents	Volume
1	1KB Ladder	5µl
2		
3	J23108 J06702 pSB1C3 SPcut	5µl
4	J23100 J06702 pSB1C3 EPcut	5µl
5	51010 pSB2K3 EScut	5µl
6		
7		

Results: Bands appeared at expected sizes.

Conclusion: Restriction digest enzymes were successful

20170627 blunt end cloning of melanin and zeaxanthin constructs into pJET

Names: Chaeli, Nimaya, Erin, Sydnee
Purpose: Melanin and Zeaxanthin Blunt End Cloning into Pjet
Combine the following reagents sequentially on ice:

Component	Volume (µL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/µL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (µL)
pJET1.2/blunt cloning vector (50ng/µL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant
Notes:
Melanin and Zeaxanthin are both in g-block form
Positive control used came with the pJET kit
Transformation included a negative control

Results:
-nothing grew on any of the plates
-will try again using a vortex

20170628 blunt end cloning of melanin constructs into pJET – seconds attempt

Names: Nimaya, Erin
Purpose: Melanin Blunt End Cloning into pJET to see if it works this time

Combine the following reagents sequentially on ice:

Component	Volume (µL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/µL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (µL)
pJET1.2/blunt cloning vector (50ng/µL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Notes:
-same procedure as last day except this time the tubes consist of only melanin, positive control (transformation, and ligation), negative control (transformation)

Results:
-nothing on any of the plates
-could be because of the cells, as not even the positive control for transformation worked. Lost competency?

20170704 blunt end cloning of melanin constructs into pJET

Names: Nimaya, Erin
Purpose: Melanin Blunt End Cloning into pJET to see if it works this time

Combine the following reagents sequentially on ice:

Component	Volume (µL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/µL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (µL)
pJET1.2/blunt cloning vector (50ng/µL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Notes:
-the tubes are using the same ligation mixture from last time (melanin) and a positive control (pUC19), the cells are new though, which might change the results
-hoping to get the positive control to work as that means the transformation is fine

Results:

-9 colonies on melanin and 87 colonies on positive control

20170705 blunt end cloning of zeaxanthin and anthocyanin constructs into pJET

Names: Nimaya, Autumn, Allison, Erin
Purpose: Zeaxanthin and Anthocyanin (DFR, 3BH and ANS) Blunt End Cloning into pJET

Combine the following reagents sequentially on ice:

Component	Volume (uL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/uL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (uL)
pJET1.2/blunt cloning vector (50ng/uL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Notes:
Tubes: Zeaxanthin, Anthocyanin DFR 3BH (front) and ANS (back), positive control ligation, negative control transformation

*for anthocyanin DFR 3BH, the pJET blunt plasmid was added before the 5 minute incubation

Results: All except the ANS back transformation had colonies

20170706 Miniprep of melA_pJET from successful pJET ligation and transformation

Names: Haroon, Nimaya, Allison, Erin

Purpose: extract plasmids from cells for further processing

Picked 3 colonies from the successful transformation and grew them up overnight in 5mL cultures.

Miniprepped whole 5 mL of each culture.

Eluted in 30µL TE buffer

Plasmid	Concentration
melA_pJET-1	333.0 ng/µL
melA_pJET-2	328.8 ng/µL
melA_pJET-3	209.1 ng/µL

20170711 Restriction digestion of miniprepped melA_pJET

Names: Nimaya, Autumn and Sydnee

Purpose: to cut approx. 500ng of plasmid DNA with two different restriction enzymes. This should provide enough cut DNA for both ligation and agarose gel electrophoresis.

Tube 1:

- Plasmid: Melanin-1 pJET
- Enzymes used: EcoRI and PstI
- Other:

Tube 2:

- Plasmid: Melanin-2 pJET
- Enzymes used: EcoRI and PstI
- Other:

Tube 3:

- Plasmid: Melanin-3 pJET
- Enzymes used: EcoRI and PstI
- Other:

Tube 4:

- Plasmid: pJET vector (positive control)
- Enzymes used: EcoRI and PstI

Expected size of products: 2000 bp insert and 2974 bp plasmid

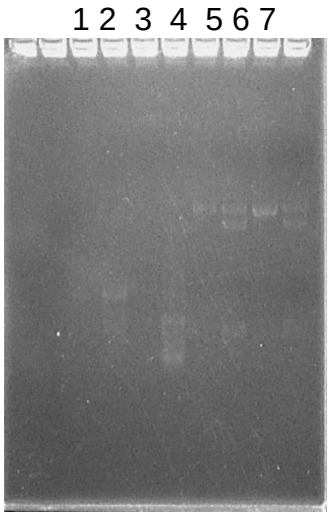
Note: Ran a PCR from another Kothe lab member (JB PCR)

Agarose gel conditions:

- Percentage: 1%
- Voltage: 100V
- Run time:_____1h_____

Lane	Contents	Volume
1		
2	JB PCR	6
3	JB PCR neg	6
4	1Kb DNA ladder	6
5	melA-1	6
6	melA-2	6
7	melA-3	6

Results:



Conclusion:

Inconclusive. Ladder did not appear well - limited size information. The JB PCR should be 830bp, which is much bigger than the small fragment we see in mel-1, which means this is likely not our insert. Will re-run gel to confirm.

20170712 Miniprep of ANS-pJET and zeaxanthin_pJET grown from transformed ligation

Purpose: harvest plasmid of ligations that were successfully transformed for further screening
Names: Nimaya and Erin

Precultures:

- Anthocyanin ANS_pJET - 1 ---culture only partially grown
- Anthocyanin ANS_pJET-2 --- no growth
- Zeaxanthin_pJET-1 --- growth
- Zeaxanthin_pJET-2 --- growth
- Zeaxanthin_pJET-3 --- growth

Miniprepped all except ANS_pJET-2

Part	Concentration (ng/µL)
Zeaxanthin_pJET-1	309.4
Zeaxanthin_pJET-2	286.3
Zeaxanthin_pJET-3	131.2
ANS_pJET - 1	108.6 (had poor A260 peak and an A230 of 0.187)

ELution volume = 30µL
Used elution buffer.

All minipreps of normal concentration. There may be ethanol contamination in the ANS_pJET as indicated by the high A230.

20170712 Miniprep of successfully transformed ANS_pJET and Zeaxanthin_pJET

Names: Nimaya, Erin

Purpose: extracting plasmids

Precultures:

- ANS_pJET-1 – partly grew, not very cloudy culture, but miniprepped anyway
- ANS_pJET-2 – did not grow
- Zeaxanthin_pJET-1 – grew
- Zeaxanthin_pJET-2 - grew
- Zeaxanthin_pJET-3 - grew

Eluted miniprep in 30µL TE buffer

Plasmid	Concentration
zeaxanthin_pJET-1	309.4 ng/µL
zeaxanthin_pJET-2	286.3 ng/µL
zeaxanthin_pJET-3	131.2 ng/µL
ANS_pJET-1	108.6 ng/µL – though very poor A260 peak and A230 = 0.187

20170718 Repeat agarose gel of melA_pJET digestions

Names: Haroon, Autumn, Erin

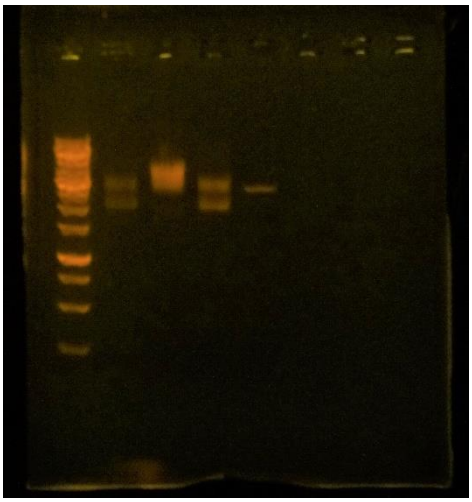
Purpose: Repetition of agarose gel to confirm melanin insertion into pJET.

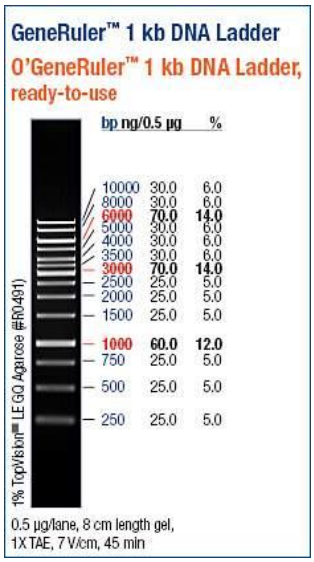
Method - Pour 1% gel that contains gel red into chamber.

Lane #	Contents	Volume
1	1KB Ladder	6µl
2	Melanin-1	6µl
3	Melanin-2	6µl
4	Melanin-3	6µl
5	Positive Control PJET alone	6µl

Ran at 100V for 40 minutes

1 2 3 4 5





Results: melA_pJET-1 and melA_pJET-3 were both successfully digested, as the produce bands appx 2000bp and 3000bp in length, which is close to what we expect for the melA insert and pJET plasmid, respectively. melA_pJET-2 was not successfully digested, indicating that there may be a problem with the restriction enzyme recognition sites. The pJET alone control is the same size as the larger band in melA_pJET-1 and -3, confirming that this band is likely pJET.

20170726 Restriction Digestion of the successful zeaxanthin and anthocyanin minipreps

Names: Nimaya, Haroon, Autumn, Candace, Erin

Purpose: to cut approx. 500ng of plasmid DNA with two different restriction enzymes. This should provide enough cut DNA for both ligation and agarose gel electrophoresis.

Tube 1:

- Plasmid: zeaxanthin-1 pJET
- Enzymes used: EcoRI and SpeI
- Other:

Tube 2:

- Plasmid: zeaxanthin-2 pJET
- Enzymes used: EcoRI and SpeI
- Other:

Tube 3:

- Plasmid: zeaxanthin-3 pJET
- Enzymes used: SpeI and EcoRI
- Other:

Tube 4:

- Plasmid: anthocyanin ANS_pJET
- Enzymes used: SpeI and EcoRI
- Other:

Expected size of products:

pJET: 2000bp

Zeaxanthin: 1000bp

Anthocyanin: 1000bp

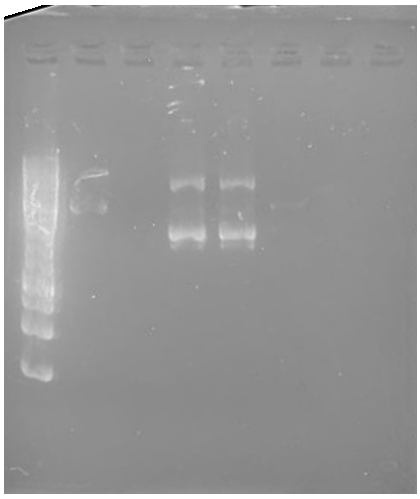
Agarose gel conditions:

- Percentage: 1%
- Voltage: 100V
- Run time: 40 minutes

Lane	Contents	Volume
1	1KB Ladder	6µl
2	pJET	6µl
3	anthocyanin pJET	6µl

4	Zeaxanthin-1 pJET	6µl
5	Zeaxanthin-2 pJET	6µl
6	Zeaxanthin-3 pJET	6µl

Results:



Conclusion:

The gel was successful, but we did not get the results that were expected (we got 2000 bp but wanted 1000 bp).

Additional Notes:

**Update: they were supposed to be 2000bp! So it was successful!!!

20170801 blunt end cloning of anthocyanin constructs into pJET

Names: Nimaya, Ally, Candace, Autumn, Erin
Purpose: Anthocyanin Blunt End Cloning into pJET

Combine the following reagents sequentially on ice:

Component	Volume (µL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/µL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (µL)
pJET1.2/blunt cloning vector (50ng/µL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Notes:
Tubes consist of anthocyanin front (DFR 3BH), anthocyanin back (ANS), anthocyanin (yadH 3GT), positive control (ligation), negative control (transformation)

Results:
No ligation was successful, though the positive control worked.

20170808 PCR Amplification of Melanin and zeaxanthin parts from pJET

Names: Nimaya and Erin

Purpose:_PCR amplification of melanin and zeaxanthin parts from pJET

Template DNA: melA_pJET-1 and Zeaxanthin_pJET-1 minipreps

Product Fragment: MelA or Zeaxanthin parts

- Expected size_appx 2000bp

Primers:

- Forward: +6 prefix
- Reverse: +6 suffix

Reagent and stock concentration	Volume	Final Concentration
MilliQ ddH2O	28.5	
10x PCR Buffer	5	1x
10 micro molar dNTPs	1	200 microM each
Forward Primer (10 micro molar)	2.5	0.5 microM
Reverse primer (10 micro molar)	2.5	0.5 microM
DNA polymerase	.5	0.02 U/uL
Template DNA	5	1-100 ng/uL

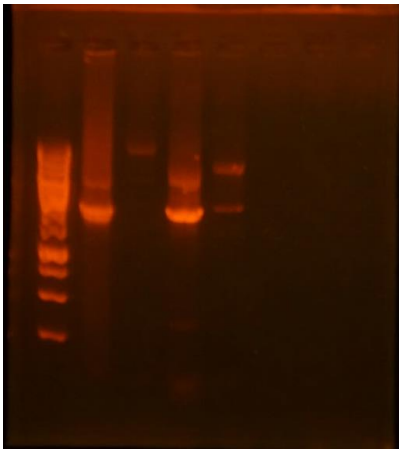
Reaction Conditions:

Initial Denaturation	95 degrees	2min	1x
Denaturation	95 degrees	30s	|
Annealing	54 degrees	30s	| 30x
Extension	72 degrees	3min	|
Final Extension	72 degrees	5min	1x
Hold	4 degrees	hold	

Agarose gel conditions:

- Percentage: _____1%_____
- Voltage: _____100V_____
- Run time: 30mins

Lane	Contents	Volume
1	Ladder	5 µL
2	Melanin positive control	6 µL
3	Melanin negative control	6 µL
4	Zeaxanthin positive control	6 µL
5	Zeaxanthin negative control	6 µL
6		



Conclusion: both the melA and zeaxanthin crtY-crtZ inserts were successfully amplified.

20170817 Restriction digestion melA_pJET, zeaxanthing_pJET and RFP_pSB1C3

Names: Nimaya, Autumn, Haroon, Brian

Purpose: to cut approx. 500ng of plasmid DNA with two different restriction enzymes. This should provide enough cut DNA for both ligation and agarose gel electrophoresis.

Tube 1:

- Plasmid: Melanin-1 pJET (1)
- Enzymes used: EcoRI and PstI
- Other:

Tube 2:

- Plasmid: Melanin-1 pJET (2)
- Enzymes used: EcoRI and PstI
- Other:

Tube 3:

- Plasmid: Zeaxanthin-1 pJET (1)
- Enzymes used: EcoRI and PstI
- Other:

Tube 4:

- Plasmid: Zeaxanthin-1 pJET (2)
- Enzymes used: EcoRI and PstI
- Other:

Tube 5:

- Plasmid: pSB1C3 (1)
- Enzymes used: EcoRI and PstI
- Other:

Tube 6:

- Plasmid: pSB1C3 (2)
- Enzymes used: EcoRI and PstI
- Other:

Additional Notes:

-this was followed by a PCR Clean up the next day to purify

Results:

Concentrations:

pSB1C3 (1) : 34.70 µg/mL

A230: 0.091A

A260: 0.052A

A280: 0.033A

A320: 0.017A

A260/A230: 0.471

A260/A280: 2.210

pSB1C3 (2): 19.01 µg/mL

A230: 0.080A

A260: 0.033A

A280: 0.022A

A320: 0.014A

A260/A230: 0.288

A260/A280: 2.374

zeaxanthin (1): 15.95 µg/mL

A230: 0.041A

A260: 0.021A

A280: 0.012A

A320: 0.005A

A260/A230: 0.444

A260/A280: 2.295

20170823 Ligation of melA-1 and zeaxanthin-2 into pSB1C3

Names: Nimaya, Harland

Purpose: Ligate the melanin and zeaxanthin parts into pSB1C3

Fragment:

Melanin

- Size: 2096 bp

Zeaxanthin

- Size: 1972 bp

Plasmid: pSB1C3

- Size: 2070 bp

Volumes	*Zea low*	*Zea high*	*melA low*	*melA high*
Vector	0.29	1.44	0.29	1.44
Insert	0.66	3.32	0.24	1.21
Buffer	1	1	1	1
T4 DNA Pol	0.5	0.5	0.5	0.5
H_2O	7.55	3.74	7.97	5.85

Confirm success of ligation by transformation, miniprep and restriction digestion.

20170824 transformation of melanin and zeaxanthin pSB1C3 ligations into *E. coli* DH5α

Names: Autumn, Nimaya, Haroon

Purpose: Transformation of melanin and zeaxanthin parts in E. coli DH5alpha

Part name(s): melanin-low pSB1C3, melanin-high pSB1C3, zeaxanthin-low pSB1C3, zeaxanthin-high pSB1C3, positive control (pSB1C3: rfp)

Plasmid description:

- Size: _
- Resistance: Cam
- Other (inserts, components, origin, notes):

Cell Strain: DH5a_

Positive Control: pSB1C3: rfp_

Negative Control:

Results:

Number of colonies on:

- Experimental:____0____
- Positive control:_____
- Negative control:____0___

Conclusion:

Only colonies spotted on positive control. None on the other plates. Most likely due to the low concentrations of the ligations.

20170830 PCR Amplification

Names: Nimaya, Harland

Purpose: PCR amplification of melanin and zeaxanthin parts from pJET

Template DNA: melA pJET-1, melA pJET -2, Zeaxanthin pJET-2, Zeaxanthin pJET-3 minipreps

Product Fragment: MelA or Zeaxanthin parts

- Expected size appx 2000bp

Primers:

- Forward: +6 prefix
- Reverse: +6 suffix

Reagent and stock concentration	Volume	Final Concentration
MilliQ ddH2O	28.5	
10x PCR Buffer	5	1x
10 micro molar dNTPs	1	200 microM each
Forward Primer (10 micro molar)	2.5	0.5 microM
Reverse primer (10 micro molar)	2.5	0.5 microM
DNA polymerase	.5	0.02 U/uL
Template DNA	5	1-100 ng/uL

Reaction Conditions:

Initial Denaturation	95 degrees	2min	1x
Denaturation	95 degrees	30s	|
Annealing	54 degrees	30s	| 30x
Extension	72 degrees	3min	|
Final Extension	72 degrees	5min	1x
Hold	4 degrees	hold	

Agarose gel conditions:

Purification Method?

- PCR clean up
-

PCR Clean Up:

Elution volume: 45µL

Elution Solution: €Water

Plasmid	Concentration (ng/µL)
Melanin -1	209.4
Melanin -1	190.4
Zea -1	14.69
Zea-2	165.2

Used directly in an EcoRI and PstI restriction

- Added 5µL 10x cutsmart buffer
- Added 1µL EcoRI and 1µL PstI
- Also digested an RFP_pSB1C3 plasmid
- Details next page…

20170831 PCR amplification of Mel and Zea, followed by digestion and ligation into pSB1C3

Names: Nimaya, Kelly, Harland
Purpose: move Mel and Zea parts into pSB1C3

Reagents	5.5x vol (uL)
MilliQ H2O	156.75
10x buffer	27.5
10mM dNTPs	5.5
+6 prefix (10mM)	13.75
+6 suffix (10mM)	13.75
MgSO4	27.5

Aliquotted 44.5uL master mix, added 5uL pJET template DNA and 0.5uL Pfu DNAP
One no enzyme negative control

Insert gel picture! Haroon!!!

EK Cleaned up PCRs, digested with EcoRI and PstI
EK digested RFP_pSB1C3 as above, dephosphorylated (added 1uL SAP, incubate 1h at 37 degrees, heat killed at 65 degrees 15 min).

Part/plasmid	A260	ng/uL
mel-1	0.133	133.4
mel-2	0.119	119.2
zea-2	0.012	11.16
zea-3	0.126	118.5
pSB1C3	0.213	191.3

Did not continue with Zea-2

Used remaining parts in a ligation reaction

Reagent	Mel1 (uL)	Mel2 (uL)	Zea3 (uL)
MilliQ	14.74	14.58	14.57
10x buffer	2	2	2
pSB1C3	1	1	1
insert	1.26	1.42	1.43
T4 DNA ligase	1	1	1

NOTE: should have done a ⅛ dilution of the pSB1C3 but forgot. LIkely too much vector in the reaction

Incubated overnight at RT

20170905 blunt end cloning anthocyanin constructs into pJET

Names: Nimaya, Allison, Erin
Purpose: Anthocyanin (DFR 3BH, ANS, 3gt yadH) Blunt End Cloning into Pjet

Combine the following reagents sequentially on ice:

Component	Volume (uL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/uL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (uL)
pJET1.2/blunt cloning vector (50ng/uL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Notes:
Tubes: Anthocyanin DFR 3BH (front) and ANS (back) and 3gt yadH, positive control ligation,

Results:
Only the positive control grew colonies. No other growth.

20170905 Ligation of melA and zea parts into pSB1C3

Names: EK

Purpose: introduce melA and crtY-crtZ constructs into pSB1C3 for submission

Combined the following:

Reagent	**melA-1**	**melA-2**	**Zea-3**
MilliQ	14.74	14.58	14.57
10x Buffer	2	2	2
pSB1C3 (1/8 dil)	1	1	1
Insert	1.26	1.42	1.43
T4 ligase	1	1	1

** no more than 100ng DNA in a 10µL reaction!

Also did a no insert negative control

Incubated at RT overnight

20170906 – transformed into DH5α added 2µL reaction to 20µL competent cells

Results: no colonies

20170908 Repeat transformation of melA and zea ligations into pSB1C3

Repeated as per standard protocol, used 1µL of ligation reaction instead of 2 µL

Parts:

- Zea-3_pSB1C3
- melA-1_pSB1C3
- melA-2_pSB1C3
- positive control pSB1C3

20170910 Miniprep of melA_pSB1C3

Names: Nimaya, Kyle, Harland

Per standard protocols, eluted in 30µl TE buffer

PART	*CONCENTRATION (MG/ML)*	*A260*	*A260/A280*
RFP_pSB1C3	181.3	0.186	1.922
Zeaxanthin pJET-3	45.18	0.102	0.958
Zeaxanthin pJET cut	116.7	0.139	1.346
Zeaxanthin pJET cut and clean 2	28.27	0.033	1.547
Zeaxanthin pJET cut and clean 1	15.08	0.016	0.396
3gt-yadH pJET ligation 20170801	635.1	0.669	2.116
3gt-yadH pJET ligation 20170905	555.0	0.587	2.071
Anthocyanin back pJET ligation	555.4	0.589	2.039
Anthocyanin from pJET ligation	832.5	0.873	2.200
pSB1C3 clean1	43.20	0.047	1.946
pSB1C3 clean2	4.322	0.006	1.861
melA_pJET 3	108.5	0.114	1.199
melA_pJET 2	150.6	0.156	1.314
melA_pJET 1	137.2	0.150	1.151
melA-1 clean	88.75	0.092	1.821
melA pJET-2 cut and clean	19.47	0.003	2.056

2017 0913 PCR of melanin, anthocyanin and zeaxanthin parts

Names: Autumn, Harland

Performed a PCR of the following parts:

- melanin-1
- melanin-2
- zea-1
- zea-2
- ans-1 (3gt yadH)
- ans-2 (and front)
- ans-3 (and-back)

Component	*Volume (µL)*
Milli Q	28.5
!0x buffer	5
10mM dNTPs	1
+6 prefix primer	2.5
+6 suffix primer	2.5
Taq DNAP	0.5
Template DNA	5*
$MgCl_2$	5

- 1uL template DNA for ans 1,2 and 3
- Adjusted for water accordingly

20170915 Ligation of DFR, 3gt-yadH and indB into pJET

Combine the following reagents sequentially on ice:

Component	Volume (uL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/uL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (uL)
pJET1.2/blunt cloning vector (50ng/uL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Results:

- DFR- 1 unhealthy looking colony
- 3GT-yadH – 2 colonies
- indB – 3 colonies

picked colonies to miniprep

20190916 – miniprep of colonies from 20170915

- only 1 colony of the 3gt-yadH transformation grew
- no growth of DFR culture
- 1 colony of indB grew

Results:

Eluted in 50µL water

indB_pJET – 152.7 ng/µL

3gt-yadH_pJET – 120.2 ng/µL

20170929 – Test production of melanin from melA_pJET in *E. coli* BL21(DE3)

Purpose; grow test cultures under various condiditon to see if we can produce melanin

Names: Kelly, Erin

Protocol:

- melA_pJET was previously transformed into *E. coli* BL21(DE3)
- large colonies were picked and used to inoculate 5mL cultures
- 6 tubes were prepared as follows:

Tube	*Amp*	*Tyrosine (5µL of 400mg/L soln)*	*$CuSO_4$ (5µL of 20mg/mL soln)*	*IPTG*
1	✓	✗	✗	✗
2	✓	✗	✗	✓
3	✓	✓	✓	✗
4	✓	✓	✗	✓
5	✓	✗	✓	✓
6	✓	✓	✓	✓

Inoculated and grown at 30 degrees, 200rpm

Induced at OD600 ~1

Grew for 3 days, replaced amp, tyr

Results: no change in OD due to melanin production, no visible black.brown colour production

20191004 Overexpression of melA from 4 colonies

Names: Allison, Nimaya, Erin

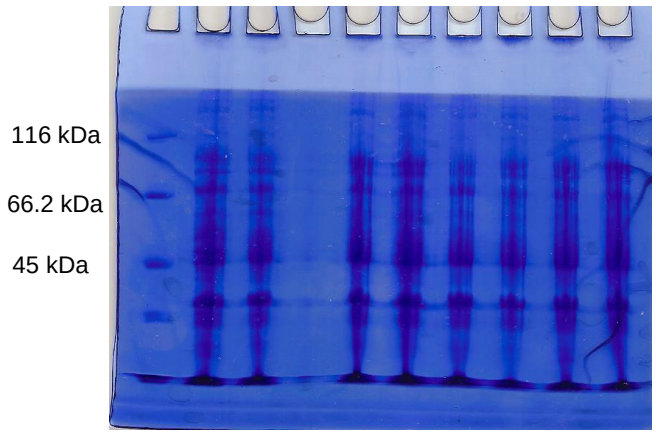
Purpose: determine if melA is being expressed

Protocol:

- Inoculated 4 cultures of BL21(DE3) melA_pJET from 4 clones, grew ON aat 35 degrees, 225 rpm shaking
- Used 200µL pre-culture to inoculate 5mL cultures
  - Grew at 37 degrees for 4h to OD600 ~1
  - Induced with IPTG, added Tyr and CuSO4 as above
  - Took 1OD sample, grew at 30 degrees fro 5 hours
  - Took 1OD sample at 5 hours
  - Added 80µL 100mM tris pH 8.5 5M urea to the cell pellets
  - Added 20µL 6x SDS Laoding Dye
  - Boiled 3 min, spin down, load on 12% SDS PAGE

Lane	*Contents*	*Volume (µL)*
1	MW ladder	10
2	MelA-1 t0	20
3		
4	MelA-1 t5	20
5	MelA-2 t0	20
6	MelA-2 t5	20
7	MelA-3 t0	20
8	MelA-3 t5	20
9	MelA-4 t0	20
10	MelA-4 t5	20

Ran at 200V 1h, stained overnight, destained overnight, expected size of melA tyrosinase is 50kDa



Conclusion: bands in overexpression sames are a bit smeary, difficult to tell individual bands apart. There may be a 50kDa band forming from cultures1 and 2 is we compare intensities in the 50kDa area in these lanes compared to cultures 3 and 4.

20172021 blunt end cloning into pJET

Purpose: clone fresh g blocks into pJET and mutations were found by sequencing previously cloned parts

Names: Nimaya, Harland

Combine the following reagents sequentially on ice:

Component	Volume (uL)
2x reaction Buffer	10
DNA fragment (g-block) (50ng/uL)	1
Nuclease free water	6
DNA blunting enzyme	1
Total Volume	18

Vortex and centrifuge for 3-5s
Incubate the mixture at 70 degrees C for 5 min and chill on ice

Set up the ligation reaction on ice. Add the following to the blunting reaction mixture.

Component	Volume (uL)
pJET1.2/blunt cloning vector (50ng/uL)	1
T4 DNA ligase	1
Total volume	20

Vortex briefly and centrifuge 3-5s. Incubate at room temperature for 5 min. Note for DNA fragments in excess of 3kb, ligation can be prolonged to 30 mins (no longer!). This ligation mixture can then be used directly for a transformation. pJET is ampicillin resistant

Took Spec readings of the parts:

Part	*Concentration (µg/mL)*	*A260*	*A260/A280*
melA	57.69	0.059	1.821
Anthocyanin – DFR	35.82	0.040	1.807
Anthocyanin – 3GT-YadH	81.96	0.081	1.823
zeaxanthin	98.41	0.105	1.843

20171022 of indB, melA-1, melA-2 and melA-3

Names: Nimaya, Harland

Spec readings:

Part	Concentration (µg/mL)	A260/A280
melA-pJET 1	129.0	1.950
melA-pJET 2	87.17	1.930
melA-pJET 3	115.8	1.905

PCR mixture:

Component	Volume (µL)
milliQ	33
5x buffer	10
BBS +6	1.5
BBP +6	1.5
Template DNA	2.5
Phusion DNAP	0.5

Results:

PCR not successful

20171025 colony PCR of indB_pSB1C3 and melA_pSC1C3

Names: Ally, Harland

Purpose: confirm insertion of parts into pSB1C3

PCR Mix:

Component	*Volume (µL)*
Template	1
Taq	1
Taq buffer	2.5
F primer BBP	0.5
R primer BBS	0.5
dNTPs	0.5
Water	19

Program:

95°C	3 min
95°C	|
58°C	| 30 cycles
72°C	|
72°C	10 min
4°C	hold

Results: PCR successful for melA! Send parts to registry!

20171029 Overexpression of melA tryrosinate

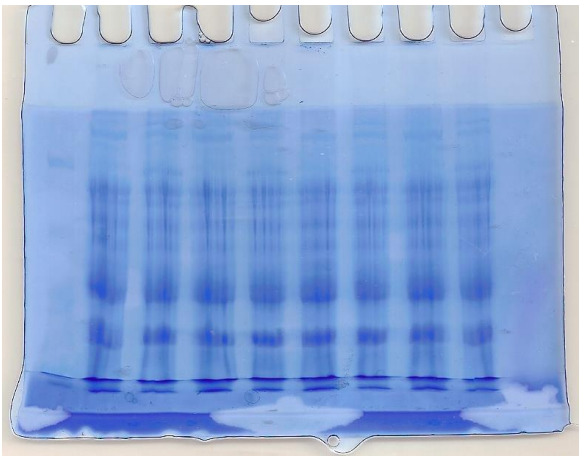
Purpose: determine if melA is being expressed

Protocol:

- Inoculated 4 cultures of BL21(DE3) melA_pJET from 4 clones, grew ON aat 35 degrees, 225 rpm shaking
- Used 200µL pre-culture to inoculate 5mL cultures
    - Grew at 37 degrees for 4h to OD600 ~1
    - Induced with IPTG, added Tyr and CuSO4 as above. Made fresh IPTG for this overexpression
    - Took 1OD sample, grew at 30 degrees fro 5 hours
    - Took 1OD sample at 5 hours
    - Added 80µL 100mM tris pH 8.5 5M urea to the cell pellets
    - Added 20µL 6x SDS Laoding Dye
    - Boiled 3 min, spin down, load on 12% SDS PAGE

Lane	*Contents*	*Volume (µL)*
1	MW ladder	10
2	MelA-1 t0	20
3	MelA-1 t5	20
4	MelA-2 t0	20
5	MelA-2 t5	20
6	MelA-3 t0	20
7	MelA-3 t5	20
8	MelA-4 t0	20
9	MelA-4 t5	20
10		

Ran at 200V 1h, stained overnight, destained overnight, expected size of melA tyrosinase is 50kDa.



Still no obvious overexpression of MelA at 50kDa.

20171030 – Making "ink" from tablets

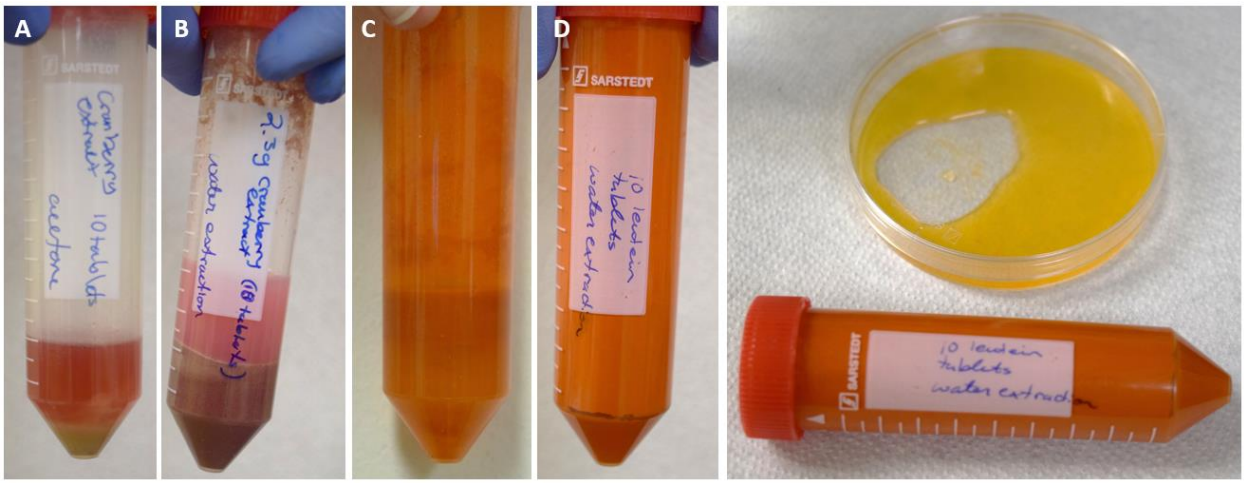
Purpose: make a proof of concept ink

Used 10 lutein tablets from Equate (each tablet contains 20mg lutein and 850µg zeaxanthin) or 10 cranberry extract tablets form Equate which contain anthocyanin.

Opened the tablets into falcon tubes and added 10mL of 80 degree water or acetone, let in in 80 degree water bath 10 min, let sit room temperature overnight

A red liquid phase was formed in both the acetone and water extractions of the cranberry tablets (panel A and B).

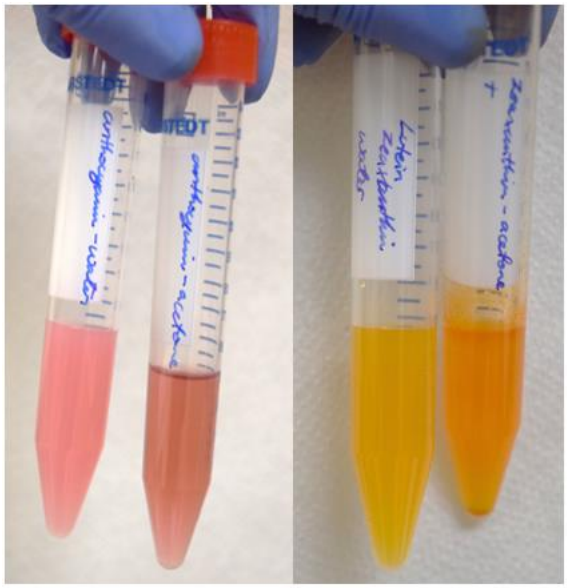
A yellow and orange phase was formed in the lutein acetone extraction, and a lighter orange yellow phase was formed in the water extraction (panel C and D).



In both cases, the acetone brought out a more vibrant colour.

Each of the liquids was separated from the solid matter and guar gum was added to a final concentration of 0.5%. Samples were left at room temp overnight to set.

Guar gum successfully thickened the water extractions, but precipitated in the acetone, leaving the liquid thin.



A Syringe was used to streak the “inks” onto Whatman paper. The acetone solutions appear to bleed more than the water solutions. The anthocyanin water ink has a more vibrant colour on paper than the anthocyanin acetone ink, opposite to what we see in the tube. The zeaxanthin acetone ink is more vibrant that the zeaxanthin water ink.