Picking Colonies

- Date: June 1, 2014
- Names: Christina, TDang, Sunny, Roya and Yoyo

- Objective: Yesterday's (May 31, 14) transformations of J04500-K331007-RFP-DT were successful on the KAN plates, so each KAN Plate of the two transformation were picked 3 times to later be miniprepped.

- Use 6 5 mL LB media tubes and work under open flame. Pick 3 X for each plate of KAN.
- Picked with 1 P2, dropped pipette tip into the 5 mL of sterile LB media.
- ** ADD 5 µL of KAN antibiotics to each 5 mL LB Tube
- ** Left in RNA Common Room overnight at 37˚C shaking

Miniprep of K331007-RFP-DT

- Date: June 1, 2014
- Names: Christina, Tdang, Sunny, Roya and Yoyo

- Objective: Yesterday's picked colonies of the J-K7-RFP-DT transformations (May 26, 2014) were miniprepped to increase stocks of DNA and later to run a gel to see if the ligations were successful.

UV Spectrophotometer –Determining DNA Concentration

- Date: June 1, 2014
- Names: Christina, Tdang, Sunny and Yoyo

- Determine the concentrations of the minipreps done earlier today
- **All tubes of K7-RFP-DT have AMP resistance
- 1/50 dilution used (50 µL of MilliQ Water and 1 Christina, Tiffany, Sunny, Kieran of DNA)

Tube	DNA	260 nm	280 nm	Concentration (ng/µL)
1	K331007-RFP-DT (IA)	0.053	0.036	135.15
2	K331007-RFP-DT (IB)	0.073	0.043	186.15
3	K331007-RFP-DT (HA)	0.043	0.030	109.65
4	K331007-RFP-DT (HB)	0.039	0.022	99.45

PCR of J04500-K331007-RFP-DT

- Date: June 3, 2014
- Names: TDang and Kieran

- Objective: To amplify the amount of J04500-K331007-RFP-DT DNA for later use

- Multiply volume by 7 in order to ensure enough of master mix
- * Protocol of PCR was taken from page 96
- PCR of May 31, 14 Transformations were each picked 3 times for a total 6 tubes as there were 2 plates of successful transformation
- * Used preset Thermocylcer setting and left in cycler

Master Mix Components	Final Volume (µL)
Milli Q H2O	103.6
10x Biobasic TAQ Reaction Buffer	17.5
10mM dNTPS	3.5
10m Biobasic MgCl2	14
10mM primer 1	14
10mM primer 2	14
5 u/µL Biobasic TAQ Polymerase	1.4

PCR of J04500-K331007-RFP-DT (continued)

Date: June 3, 2014
Names: TDang and Kieran

DNA	
KAN 1.1	Picked from KAN 1x Transformation for J-K7-RFP-DT
KAN 1.2	“ ”
KAN 1.3	“ ”
KAN 2.1	Picked from KAN 2x “ ”
KAN 2.2	“ ”
KAN 2.3	“ ”

Digestion of J04500 and K331007-RFP-DT

- Date: June 3, 14
- Names: Christina, TDang, Sunny and Kieran

- Objective: Previous ligation of J04500-K331007-RFP-DT were screened bad so were are digesting J04500 and K331007-RFP-DT

- **Protocol taken from pg. 57
- Incubate at 37˚C in E770 overnight

J04500	
Components	Volume (µL)
Milli-Q H2O	9
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	8
Spec1	0.5
Pst1	0.5
Total	20

K331007-RFP-DT (IA from June 1)	
Components	Volume (µL)
Milli-Q H2O	9
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	8
Xba1	0.5
Pst1	0.5
Total	20

Digestion of J04500 and K331007-RFP-DT

- Date: June 4, 14
- Names: Wesley, Ronja, Tiffany Dang, Tiffany Trinh, Zak

- Yesterdays restriction failed, we are doing it again.
- Tubes:
  - J04500 (cut at S and P) R.K, T.D, T.T June 4,2014
  - K7-RFP-DT (cut at X and P) R.K, T.D, T.T June 4, 2014
- Incubate at 37°C in E770 overnight

J04500	
Components	Volume (µL)
Milli-Q H2O	9
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	8
Spec1	0.5
Pst1	0.5
Total	20

K331007-RFP-DT (IA from June 1)	
Components	Volume (µL)
Milli-Q H2O	9
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	8
Xba1	0.5
Pst1	0.5
Total	20

CFU Assay

- Date: June 5, 2014
- Names: Zak, Ronja, Tiffany Trinh, TDang and Wes

- Amp added @ 5:20
- For the OD readings: \X Amp = 100 ng/µL
- At each time point, for each flask dilutions of the liquid culture were made (1/10, 1/100, 1/1000) and plated pm LB Agar with AB
- Plates put in 37˚C incubator in E770 at 11:00 pm

OD Readings					
Time	1000X	100X	10X	1X	NA
4:40	0.295	0.283	0.305	0.270	0.361
6:20	0.120	0.939	0.965	0.993	1.063
8:25	0.120	1.145	1.213	1.225	1.354
9:30	0.120	1.358	1.445	1.446	1.479

Liquid Culture Conditions		
Flask	Antibiotic Added	Final Concentration
1000X	50 µL 1000X AMP	1X
100X	50 µL 100X AMP	0.1X
10X	50 µL 10X AMP	0.01X
1X	50 µL 1X AMP	0.001X
No AMP	No AB	NA

1% Agarose Gel

- Date: June 5, 2014
- Names: Yoyo

- Objective: To obtain the K331007 gene of interest from previous ligations (see page 109)

- Method: 1% Agarose gel with 20 mL 1X TAE and 0.2 g Agar A
- Ran for 135V for 30 mins
- Stained in EtBR for about 15 mins
- Destained for about 30 mins

Lane	
1	I KB Ladder (Truen Science)
2	K331007 (20 µL + 2 µL Dye)

Gel Extraction

- Date: June 5, 2014
- Names: Yoyo

- **Be sure to use the long wave UV lamp
- 1.5 mL microfuge tube
  - Before (g) = 0.98
  - After (g) = 1.09
  - Gel (g) = 0.1 g =110 mg
- 110 mg X 4 = 440 µL of Binding Buffer II
- Followed protocol on oage 42 for purification

J04500 PCR Cleaning

- Date: June 5, 2014
- Names: Yoyo

- Objective: Get ride of buffers, enzymes, etc. in J04500 restriction tube
- Followed protocol on page 8 of BioBasic EZ-10 Column Handbook
- **Eluted with 60˚C H20, 40 µL

Nanodrop of K331007 Gel Extract and J04500 Cleanup (Followed by a ligation of K331007 Gel Extract + J04500 Clean Up)

- Date: June 5, 2014
- Names: Zak

- Nanodrop Concentration
  - K331007: 5 ng/µL in 40 µL
  - J04500: 35.6 ng/µL in 40 µL
- Rules
  - Ratio 3:1 Insert (K331007) to Vector (J04500)
  - Need 105 g of K7 for each V of J04500
  - Rotovaping to 10 µL (20 ng/µL)
    - Add about 5 µL of K7 after rotovap
- Made 2 ligations. One at room temperature in E770 and one in black cycler in Widen Lab. JK7 16 or JK7 RT.

Component	Volume (µL)
Milli-Q H2O	7
10X Ligase Buffer	1.5
DNA Insert (K7-RFP-DT)	5
DNA Vector (J04500)	1
T4 Ligase	0.5
Total	15

Transformation of June 5 Ligations

- Date: June 6, 2014
- Names: Zak and Christina

- Ligations run at 16˚C and room temperature for 16 hours
- Transforming 2 cultures each
  - 16˚C -AMP
  - 16˚C -KAN
  - Room Temp -AMP
  - Room Temp -KAN

- Added 1.5 µL of ligation to 20 µL competent cells
- Incubate on ice for 30 mins
- Heat shock at 42˚C for 45 seconds
- Incubate on ice for 5 mins
- Added 400 µL sterile LB media to each tube
- Shake at 37˚C for 1 hour
- Plate 200 µL on plate with correct resistance
- Incubate overnight at 37˚C

Restriction of RFP-DT, K8 and K9

- Date: June 6, 2014
- Names: Zak and Christina

- From page 84 of lab book for reference

2 X K8 = 192 ng/µL
1 X K9 = 190 ng/µL
1 X RFP-DT = 116 ng/µL

- Put in E770 37˚C incubator at 6:20 pm
- DNA put in Zak's E7770 -20˚C box

Components	Volume (µL)
H2O (MilliQ)	10
10x CutSmart Buffer	2
DNA	6
Enzyme 1	1
Enzyme 2	1
Total	20

PCR of June 6 Transformations

- Date: June 8, 2014
- Names: Tdang and Yoyo

- Objective: The transformations of the full construct of J04500-K331007-RFP-DT seemed to fail as no red colonies grew. The J04500 likely did not attach but we will run a PCR to confirm

- We are picking 3 times each on the AMP/KAN plates of the June 6 transformation along with 2 times on the RNA plate Zak gave us. The master mix was multiplied by 9 to ensure we have enough.
- **We also made a giant dilute 10mM stock of $MgCl_2$ (144 µL of MilliQ + 36 µL of $MgCl_2$)
- Also giant (about 200 µL) dilution of prefix and suffix primers (20 µL Primer and 180 µL Milli Q)for future colony PCRs
- **All plates from June 6

Master Mix Components	Final Volume (µL)
Milli Q H2O	103.6
10x Biobasic TAQ Reaction Buffer	17.5
10mM dNTPS	3.5
10m Biobasic MgCl2	14
10mM primer 1	14
10mM primer 2	14
5 u/µL Biobasic TAQ Polymerase	1.4

PCR of June 6 Transformations

- Date: June 8, 2014
- Names: TDang and Yoyo

P1	P2	P3	P4	P5	P6	P7	P8
AMP/KAN J04500-K331007-RFP-DT #1	"#2"	"#3"	AMP/KAN J04500-K331007-RFP #1	"#2"	"#3"	RNA Out Synet Zak #1	#2

- Ran program Zak > #4 > PCR
- Annealing temp 78˚C
- Extension 1 min 30 sec for 35 cycles

1% Agarose Gel

- Date: June 8, 2014
- Names: Yoyo

- Objective: Run the colonies PCR on a 1% gel to see if the previous ligations worked

- Ran for 45 mins @ 100 V
- Stained in EtBr for about 15 mins
- De-stained for about 30 mins
- Colony PCR failed

Lane	
1	1 KB Ladder (Truin Science about 10 µL)
2	RFP-DT 1/100 Dilution Control (about 18 µL + 2 µL Dye)
3	C1 J-K7-RFP-Dt #1 Plate #1 (about 18 µL + 2 µL Dye)
4	C2 " " #2 " "
5	C3 " " #3 " "
6	C4 J-K7-RFP-Dt #1 Plate #2
7	C5 " " #2 " "
8	C6 " " #3 " "
9	C7 RNA Out Synth Zak #1
10	C8 " " #2
11	RFP-DT 1/100 Dilution Control
12	1 KB Ladder (Truin Science about 10 µL)

1% Agarose Gel

- Date: June 8, 2014
- Names: Yoyo

- Objective: Run a 1% agarose gel to extract the gene of interest of June 6 digest (k8, K9, RFP-DT)

- Ran for 45 mins @ 100V
- Stained in EtBr for about 15 mins
- De-stained for about 30 mins

Lane	
1	1 KB Ladder (Truin Science about 10 µL)
2	RFP-DT (Cut X, P) (20 µL + 2 µL Dye)
3	K331008 (Cut S, P)
4	K331009 (Cut S, P)

Gel Extraction and Purification

- Date: June 8, 2014
- Names: Yoyo

- Tubes temporarily in -4˚C in Wieden lab
- Gel extraction eluded in 30 µL
- Concentrations
  - K8 -16.8 ng/µL
  - K9: 21.9 ng/µL
  - RFP -20 ng/ µL

Tube #	Before (g)	After (g)	Gel Weight	Binding Buffer II (µL)
Tube 1 RFP-Dt (Cut X, P)	0.94	1.07	0.13 → 130 mg	520
Tube 2 K8 (Cut S, P)	0.95	1.04	0.09 → 90 mg	360
Tube 3 K9 (Cut S, P)	0.97	1.02	0.05 → 50 mg	200

Ligation of K8/K9 and RFP-DT

- Date: June 9, 2014
- Names: Zak

- Ligation Rules
  - 3:1 insert to vector
  - < 100 ng/µL final
- Total RFP volume = ≈ 28 µL -14 µL each (288.4 ng)
- Add 96.1 ng pf K8 (5.7 µL) and K9 (4.4 µL)

Reaction Mix	K8 (µL)	K9 (µL)
MilliQ	2.2	3.5
10X Ligase Buffer	2.5	2.5
RFP-Dt (Insert)	14	14
KX (Vector)	5.7	4.4
T4 Ligase	0.6	0.6
Total	25	25

Restriction/Digest

- Date: June 9, 2014
- Names: Yoyo

- Objective: Restrict more J04500 and K331007-RFP-Dt for more ligations … back-up

- ** J04500 cut with S, P
- ** K7-RFP-DT cut with X, P
- Tubes (x4) in E770 37˚C incubator @ 6:00 pm

Components	Volume (µL)
H2O (MilliQ)	10
10x CutSmart Buffer	2
DNA	6
Enzyme 1	1
Enzyme 2	1
Total	20

CFU Assay Round 2

- Date: June 10, 2014
- Names: Zak, TDang, Yoyo and later Kieran

- Culture (WT DH5α)

C1V1=C2V2
1.934 V1 = 0.1(5 mL)
X = 0.5 (OD 600 µL) / 1.934 (OD 600 µL)
X = 0.2585 µL = 258.5 µL

- Take out 258.5 µL from, each tube of LB
- Add 258.5 µL cells to culture tubes
- 6 AMP Concentrations
- 3 time points
- 3 dilutions
- Results in 54 plates
- Plating Dilutions 1/100, 1/10 000, 1/ 1 000 000

CFU Assay Round 2

- Amp Concentrations
  - 100 µg/µL
  - 80 µg/µL
  - 60 µg/µL
  - 40 µg/µL
  - 20 µg/µL
  - 0 µg/µL

Dilution Concentrations

For 80 µg/µL
C1V1 = C2V2
100 µg/µL V1 = (80 µg/µL) (10 µL)
V1 = 8 µL of antibiotics

For 60 µg/µL
V1 = 6 µL of antibiotics

For 40 µg/µL
V1 = 4 µL of antibiotics

For 20 µg/µL
V1 = 2 µL of antibiotics

CFU Assay Round 2

- Date: June 10, 2014
- Names: TDang and Yoyo

- At 5:45 pm
  - 1 mL of the O/N cultures was taken
  - 4 µL of AMP Dilutions added to their respective labeled LB flasks (starting with 100 µg/µL)
- 600 µL culture samples were taken at each time point
- 500 µL was mixed with 500 µL LB and OD600 readings taken
- Rest used for serial dilution
- Each culture diluted by 100, 10 000, 1 000 000 tubes and each dilution was plated on LB Agar with no antibiotics
- Dilutions
  - 50 µL of culture added to 4950 µL of LB
  - 2 µL of 1/100 added to 198 µL of LB
  - 2 µL of 1/10 000 added to 198 µL of LB
- 200 µL of culture dilutions were plated and put in E770 37˚C incubator

CFU Assay Round 2

- Date: June 10, 2014
- Names: Zak, Yoyo, Tdang and Kieran
- These are 500 µL culture with 500 µL LB

OD Readings						
Time	100 µg/µL	80 µg/µL	60 µg/µL	40 µg/µL	20 µg/µL	0 µg/µL
6:00	0.232	0.243	0.251	0.232	0.251	0.205
6:41	0.179	0.241	0.306	0.329	0.401	0.324
7:37	Bad Reading	0.045	0.073	0.258	0.534	0.595
8:43	0.041	0.040	0.046	0.100	0.539	0.865

Counting Colonies from June 10 CFU Assay

- Date: June 11, 2014
- Names: Kieran and TDang

- Objective: The 1/100, 1/10 000, 1/1 000 000 dilutions worked as the 1/1 000 000 dilution has enough colonies that can be ACTUALLY counted so today we are counting the plates from last night's CFU assay so the data can be graphed
- Divide the plate into quarters and label the quadrants accordingly so that the colonies can be counted easier
- ** Not Countable (too many) = NC
- Chart on following slide(s)
- ** The plate with no cells and no antibiotics had no cells grow

Counting Colonies from June 10 CFU Assay

Time 1: 6:43 pm					
Concentration	Dilution	Number of Colonies in Quad			
		1	2	3	4
0 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	NC	NC	NC	NC
	1/1 000 000	22	19	15	10
20 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	NC	NC	NC	NC
	1/1 000 000	12	4	4	4
40 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	113	213	195	203
	1/1 000 000	4	7	3	1
60 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	44	51	69	110
	1/1 000 000	1	0	0	2
80 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	45	45	57	17
	1/1 000 000	0	0	0	0
100 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	79	10	1	14
	1/1 000 000	1	1	0	0

Counting Colonies from June 10 CFU Assay

Time 2: 7:37 pm					
Concentration	Dilution	Number of Colonies in Quad			
		1	2	3	4
0 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	NC	NC	NC	NC
	1/1 000 000	22	26	26	24
20 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	283	108	224	226
	1/1 000 000	4	1	2	1
40 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	377	162	147	336
	1/1 000 000	1	4	7	3
60 µg/µL	1/100	233	196	164	151
	1/10 000	1	2	3	5
	1/1 000 000	0	0	2	0
80 µg/µL	1/100	84	104	61	139
	1/10 000	2	0	0	2
	1/1 000 000	0	0	0	0
100 µg/µL	1/100	162	106	107	59
	1/10 000	0	0	0	0
	1/1 000 000	5	1	5	3

Counting Colonies from June 10 CFU Assay

Time 3: 8:43 pm					
Concentration	Dilution	Number of Colonies in Quad			
		1	2	3	4
0 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	NC	NC	NC	NC
	1/1 000 000	42	40	75	103
20 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	367	484	NC	NC
	1/1 000 000	18	19	16	16
40 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	7	12	16	19
	1/1 000 000	7	16	11	5
60 µg/µL	1/100	NC	NC	NC	NC
	1/10 000	NC	NC	NC	NC
	1/1 000 000	37	42	71	81
80 µg/µL	1/100	19	28	26	18
	1/10 000	1	0	3	0
	1/1 000 000	0	0	0	1
100 µg/µL	1/100	41	39	46	26
	1/10 000	0	2	0	0
	1/1 000 000	0	0	0	0

Restriction of All K331007-RFP-DT

- Date: June 12, 2014
- Names: Ronja, TDang and Yoyo

- Objective: On previous gels we've been getting weird results as multiple bands have been showing up instead of one clear bamd. Either our enzymes are failing (but we have been using E770) or we don't have the right part. We are going to restrict ALL of our possible K331007-RFP-DT and run a gel to see if any work.

- **After looking in the -20˚C freezer the only K331007-RFP-DT is IA, IB, HA, and HB from June 1, 2014 minipreps
- Placed in 37˚C incubator in E770 at 5:41 pm

Components	Volume (µL)
H2O (MilliQ)	4
10x CutSmart Buffer	2
DNA (K331007-RFP-DT)	2
Xbal	1
Pst 1	1
Total	10

PCR of J04500-K331007-RFP-DT

- Date: June 12, 2014
- Names: Yoyo, Christina, Roya, and TDang

- Objective: Chris earlier tried to do a colony PCR of the picked plates Zak did today. However, the annealing time was incorrect and so we are retrying the PCR.

- *We are unsure where the JO4500-K331997-RFP-DT come from.
- * Protocol taken from pg 96.
- *22 Had less mastermix than the other tubes.
- *23+24 were omitted due to inadequate amount of master mix.
- *24 tubes of PCR were completed for the PCR.

Master Mix		
Components	Initial	Final
Milli Q	14.8	384.8
10x Biobasic TAQ Rxn Buffer	2.5	65
10 mμ dNTPS	0.5	13
10 mμ Biobasic MgCl2	2	52
10 uμ Prefix Primer	2	52
10 uμ Suffix Primer	2	52
5 μ/uM BioBasic TAQ Polymerase	0.2	5.2

1% Agarose Gel

- Date: June 12, 2014
- Names: Yoyo, Roya, Christina, and TDang

- Objective: We are doing a 1% Agarose gel and running our digestions of K7-RFP-D7 from earlier today to confirm if we have the correct bands and ultimately if we have the right parts.

- DNA (IA, IB, HA, HB)
  - 10 µL DNA
  - 2 µL Loading Dye
- 1 KB Ladder
  - 5 µL 1 KB Ladder
- * Gel made that day used maybe not autoclaved tips.
- Run @ 135 V for 45 mins.
- Stain in EtBr for 25 mins
- De-stain for 40 mins

Lane	
1	1 KB Ladder
2	IA (K331007-RFP-DT)
3	IB (“ “)
4	HA (“ “)
5	HB (“ “)

1% Agarose Gel

- Date: June 13, 2014
- Names: TDang, Tiffany, Sunny, Zak and Yoyo

- Objective: Running a 20 mL 1X TAE and 50 mL TAE gel today to confirm if we have successful ligations from June 12 PCR and Zak's digests from today

- 20 mL 1X TAE
  - 0.2 g Agarose A
  - 20 mL 1X TAE
  - Initial Volume 94.17 g
- 50 mL 1X TAE
  - 0.5 g Agarose A
  - 50 mL 1X TAE
  - Initial Volume 141.42 g

1% Agarose Gel

- Date: June 13, 14
- Names: Tdang, Tiffany, Sunny, Zak and Yoyo

- For the 20 mL 1X TAE
- Zak had earlier done digests today of the plate of K331007-RFP-DT from May 9 (successful ligations) and so we are running both restrictions on a gel
- DNA
  - 5 µL DNA
  - 2 µL Loading Dye
- 1 KB Ladder
  - 5 µL 1 KB Ladder (Truin)
- Run 135 V for 35 mins
- Stain for 15 mins
- De-stain for 30 mins

Lane	
1	1 KB Ladder
2	K331007-RFP-DT (Digest A from May 9 Plate Colony)
3	K331007-RFP-DT (Digest B from May 9 Plate Colony)

1% Agarose Gel

- Date: June 13, 14
- Names: Tdang, Tiffany, Sunny, Zak and Yoyo

- For a 50 mL 1X TAE
- In addition we are running a gel for 22 tubes of our colony PCR done yesterday June 12, 14
- DNA
  - 4 µL DNA
  - 1 µL Loading Dye
- 1 KB Ladder
  - 5 µL 1 KB Ladder (Truin)
- Run 135 V for 45 mins
- Stain for 15 mins
- De-stain for 30 mins

Lane	
1	1 KB Ladder (Truin Science)
2	Colony PCR Tube 1
3	Colony PCR Tube 2
…	…
23	Colony PCR Tube 22
24	1 KB Ladder (Truin Science)

Picking Glycerol Stocks for J04500

- Date: June 13, 2014
- Names: TDang and Yoyo

- Objective: Right now we need to grow over night stocks of J04500 for dialysis tubing test

- Using a flask add 50 mL of LB media
- Work aseptically under an open flame and using the P10
- J04500 glycerol stock from E770 drop the tip into the flask
- Put the flask in the RNA Common Room to incubate overnight

Picking K331007-RFP-DT Cells

- Date: June 14, 2014
- Names: Yoyo and TDang

- Objective: Stocks of we hope successfully transformed K331007-RFP-Dt were picked from the May 7 plate in E770. If all goes well we will hopefully found 1 out of 6 pick sites to have our successful construct

- Work aseptically under an open flame
- 5 µL of AMP AB added to each 5 mL LB Tubes
- We picked 3 times from already picked pick sites in addition to 3 new colonies on the plate
- Left in 37˚C shaking incubator in the RNA Common Room overnight

Digestion of K331007-RFP-Dt

- Date: June 14, 2014
- Names: Yoyo and Tdang

- Objective: Our gel last night of the digested K331007-RFP-DT was unsuccessful as we could only see the backbone. We still have some of the successful ligations in D770 and so we are restricting it today to run and confirm if they are successful.
- Leave to incubate at 37˚C for one hour in E770

Components	Volume (µL)
H2O (MilliQ)	5
10x CutSmart Buffer	2
DNA (K331007-RFP-DT)	1
Xbal	1
Pst 1	1
Total	10

1% Agarose Gel

- Date: June 14, 2014
- Names: Yoyo and Tdang

- Objective: Check our previous digest to see if the gene of interest (K331007-RFP-Dt) is present
- Ran for 30 mins at 135 V
- Stain for about 15 mins
- De-stain for about 30 mins
- ** Only backbone seen … barely

Lane	
1	1 KB Ladder (Truin Science)
2	G: K331007-RFP-Dt + 2 µL Loading Dye
3	H: K331007-RFP-Dt + 2 µL Loading Dye
4	I: K331007-RFP-Dt + 2 µL Loading Dye

1% Agarose Gel

- Date: June 14, 2014
- Names: Yoyo and Tdang

- Objective: Double check our colony PCR from June 12. There's a possibility that only the K331007 was amplified!
- Ran for 10 mins at 100 V
- Stained and detsined
- Ladder didn't separate
- Ran for 20 min at 100 V
- Stained and destained
- Gel looks like crap … but bands at second bottom ladder bands

Lane	
1	50 bp Ladder
2	Colony PCR Tube 1
3	Colony PCR Tube 2
…	…
23	Colony PCR Tube 22
24	50 bp ladder

Transformation K331007-RFP-Dt

- Date: June 14, 2014
- Names: Yoyo

- Objective: Found some tubes with K331007-RFP-Dt successful ligations, though, not much DNA is left. Transformation to grow more. Last hope for K331007+RFP-DT ligations…

- Followed protocol of April 17th
- Step 3) only added 0.8 µl of DNA
- Step 7) & 8) change LB media volume to 100 µl
- 3 transformations: G,H,I
- In RNA common room at 10:50pm

Miniprep of K331007+RFP-DT O/N cultures from June 14

- Date: June 15, 2014
- Names: Yoyo

- Objective: Get DNA, see if it's the right part
- Followed protocol from April 13th, 2014
- Pick #1, #2 did not grow

Making Glycerol Stocks

- Date: June 15, 2014
- Names: Yoyo

- Objective: make stocks of cells for backup DNA
- Procedure:
  1. Pipette 500 µl of old culture into sterile glycerol
  2. Vortex to mix well
  3. Flash freeze with liquid nitrogen
  4. Store in -80˚C freezer in HS iGEM Box

Digest of K331007+RFP-DT

- Date: June 15, 2014
- Names: Yoyo

- Objective: small sample of digest of K331007+RFPT-DT just minipreped to find gene of interest
- Incubate for 1hr at 37˚C in E770
- *Looks like pick #3has gene of interest, can't see other bands on gel

Component (x4 tubes of 3,4,5,6)	Volume (µl)
Milli-Q Water	4
10X Cutsmart Buffer	2
DNA	2
XBa1	1
Pst1	1
Total	10

1% Agarose Gel

- Date: June 15, 2014
- Names: Yoyo

- Objective: run digest on gel, hopefully see gene of interest
- Ran for 35 mins at 135 V
- Stained and de-stained
- Lane 5 was broken

Lane	
1	1 kb Ladder
2	K331007+RFP-DT #3
3	K331007+RFP-DT #4
4	K331007+RFP-DT #6
6	K331007+RFP-DT #5

O/N Digest

- Date: June 15, 2014
- Names: Yoyo

- Objective: digest K331007+RFP-DT x4 tubes minipreped today. For future assembly
- Incubated at 37˚C in E770 O/N cultures of K331007+RFP-DT

Component (x4 tubes of 3,4,5,6)	Volume (µl)
H2O	8
Cutsmart Buffer	1
DNA	15
XBa1	0.5
Pst1	0.5

Picking Colonies from June 14 Transformation

- Date: June 15, 2014
- Names: Yoyo

- Objective: grow old cultures of K331007+RFP-DT
- Method: simple stuff…

- Three plates: G,H, I and three picks per plate: G1, G2, G3 etc.
- Grew with 5µl of AMP
- In RNA Common room 37˚C with shaking at 8:30pm

1% Agarose

- Date: June 16
- Names: Yoyo

- Objective: run over O/N restriction on a gel to separate backbone & gene of interest
- Ran for 35mins at 135V
- Stain & destain
- Pick #3 (lane 2) only good one (at ~1000bp)

Lane	
1	1 kb Ladder
2	K331007+RFP-DT cut X,P #3
3	K331007+RFP-DT cut X,P #4
4	K331007+RFP-DT cut X,P #5
6	K331007+RFP-DT cut X,P #6

Gel Extract from June 16

- Date: June 16, 2014
- Names: Yoyo

Before	After	Gel
0.95µl	1.05µl	0.10µl

- 100mg x4= 400µl Binding Buffer II
- Followed previous protocol for gel purification with some modifications
- Ez-10 initial spin at 400xg for 2mins.
- Final Spin at 12,000rpm for 2mins.

Making Cultures of J04500 Cells

- Date: June 16, 2014
- Names: Ronja, TDang, Wesley, Zak

- Objective: Tomorrow we are running the dialysis tubing test but we need AMP resistance cells in order to export the Beta lactamase produced. Already Yoyo has grown the cells in a flask of 50 mL stored in D770 at 4˚C.

- Work aseptically under an open flame
- Add 50µL of AMP AB to a flask of 50 mL LB media
- Add 50µL of cells from the flask stored at 4˚C into the erlenmeyer flask with LB media
- Incubate at 37˚C with shaking in the RNA common room

Ligations

- Date: June 16, 2014
- Concentrations
  - K7-RFP-DT = 2.8 ng/µL
  - J04500 = 15.5 ng/µL
  - pSBIC3 = 265 ng/µL
- Dehydrated + Rehydrated
  - K7RFP- 40 µL x 2.8 ng/µL = 112ng in 3µL milliQ=37.3ng/µL

Components	Volume (µL)
Milli-Q H2O	4.5
10x Buffer (T4 Ligase)	1
K331007-RFP-Dt	3
J04500	1
T4 Ligation Enzyme	0.5
Total	10

Dialysis Tubing Testing

- Date: June 17, 2014
- Names: Kieran, Zak, TDang and Yoyo

- Objective: Big component to the project. Today we are running the dialysis tubing testing to measure and see if our theory behind the project works. After we are running a CFU assay on our samples and compare it to our baseline.

- Followed the protocol in the kit for the dialysis tubing set up
  1. Remove the dialysis tuning from packaging. Firmly holding the collar of the top piece with one hand and the packaging tube with the other hand, slowly twist in opposite directions. Pull the device straight out carefully.
  2. Pre-wet the membrane first in 10% alcohol followed by thoroughly flushing and soaking in Milli Q
  3. Using a pipette carefully load the sample inside and at the bottom of the membrane
     - Our sample is the culture of J04500 grown on June 16
  4. Thread the body of the dialysis tubing through the hole in the floatation ring. Float it vertically in the dialysale reservoir containing a stir bar and adjust the stirring rate to form a gentle rotating current.
     - Reservoir is LB media with AMP
- Samples take at each hour (starting at 4:45 and for the next 12 hours)
- 6 mL samples were taken

Colony PCR to screen J+K7+RFP

- Date: June 18, 2014
- Name: Zak

- Colonies picked, added to 50µL MilliQ, heat @ 95˚C for 5min, centrifuged @ 10,000rpm for 2 mins.
- 14 reactions
  - 12 Colonies
  - J04500 from maxi
  - J04500 from Red Culture

PCR MasterMix	Volume (µL)
MilliQ	24.7
10 x TAK buffer	33
10µM dNTPs	6.6
20µM MgCl2	33
10µM VFZ	33
10µM VR	33
Polymerase	1.7

Digestion of J04500 (maxiprep dilution), K808000 (Arabinose Promoter), and K7-RFP

- Date: June 18, 2014
- Names: Zak

- **4, 1** J04500- 250 ng/µL dilution of maxiprep
- **5, 2** K808000- 672 ng/µL Miniprep
- **6, 3** K7-RFP- Yoyo miniprep
- ***Total 25µL

Specing of our Dialysis Tubing Samples (CFU)

- Date: June 18, 2014
- Names: Krista, TDang, Kieran and Zak

- Objective: Our samples of our 1 hour intervals were left in the freezer in E770. So today we are specing all our samples for our CFU Assay
- Specing Rules
  - Blank with 1 mL of LB Media
  - Each sample has 500 µL of LB Media and 500 µL of sample for each interval
- Dilution Rates
  - 1/ 100 has 50 µL of culture added to 4950 µL of LB media
  - 1/ 10 000 has 2 µL of 1/ 100 added to 198 µL of LB media
  - 1/ 1 000 000 has 2 µL of 1/ 10 000 added to 198 µL of LB media
- Plate on no AB plates however plate only the 1/ 10 000 and 1/ 1 000 000 as the 1/ 100 will have way too many colonies to even count. Store in E770 at 37˚C

Specing of our Dialysis Tubing Samples (CFU)

- No AB cells created for the OD
  - 10 X 6 mL samples from dialysis
  - 2 X 5 mL No AB cells
  - We want a starting OD of 0.1 to know how much of the cells we add to the dialysis tubing samples
  - OD Readings of no AB
    - Tube 1 = 1.293
    - Tube 2 = 1.367

$C_1V_1=C_2V_2$

$1.293\ (V_1)= (0.1)(6\ mL)$

$V1 = 0.46\ mL$

$C_1V_1=C_2V_2$

$1.367\ (V_1)= (0.1)(6\ mL)$

$V1 = 0.44\ mL$

Specing of our Dialysis Tubing Samples (CFU)

OD Readings										
Time	4:45	5:49	6:45	7:45	8:45	9:45	10:54	11:45	12:45	1:45
5:45	0.205	0.174	0.187	0.197	0.216	0.202	0.211	0.166	0.175	0.202
7:00	0.048	0.267	0.265	0.257	0.285	0.262	0.244	0.255	0.265	0.263
8:00	0.015	0.230	0.313	0.330	0.363	0.304	0.324	0.308	0.343	0.314

Counting Colonies from Dialysis Tubing Test

Time 5:45 (1/10 000 Dilution)	
Time	Number of Total Colomies
4:45	0
5:49	0
6:45	0
7:45	0
8:45	0
9:45	> 300
10:45	> 300
11:45	> 300
12:45	> 300
1:45	> 300

Counting Colonies from Dialysis Tubing Test

Time 5:45 (1/1 000 000 Dilution)	
Time	Number of Total Colomies
4:45	0
5:49	0
6:45	0
7:45	0
8:45	0
9:45	86
10:45	52
11:45	56
12:45	29
1:45	60

Counting Colonies from Dialysis Tubing Test

Time 7:00 (1/10 000 Dilution)	
Time	Number of Total Colomies
4:45	> 300
5:49	> 300
6:45	> 300
7:45	> 300
8:45	> 300
9:45	> 300
10:45	> 300
11:45	> 300
12:45	> 300
1:45	> 300

Counting Colonies from Dialysis Tubing Test

Time 7:00 (1/1 000 000 Dilution)	
Time	Number of Total Colomies
4:45	0
5:49	13
6:45	70
7:45	> 300
8:45	109
9:45	> 300
10:45	22
11:45	39
12:45	173
1:45	55

Counting Colonies from Dialysis Tubing Test

Time 8:00 (1/10 000 Dilution)	
Time	Number of Total Colomies
4:45	> 300
5:49	> 300
6:45	> 300
7:45	> 300
8:45	> 300
9:45	> 300
10:45	> 300
11:45	> 300
12:45	236
1:45	3

Counting Colonies from Dialysis Tubing Test

Time 8:00 (1/10 000 Dilution)	
Time	Number of Total Colomies
4:45	0
5:49	11
6:45	46
7:45	40
8:45	48
9:45	31
10:45	128
11:45	119
12:45	> 300
1:45	18

Loading and Running Agarose Gel

- Date: June 19, 2014
- Names: Kieran, TDang and Yoyo

- Objective: With wikifreeze tomorrow and we still need results to confirm if our ligations worked. We are running a 1% agarose gel to see if our digests from June 18, 2014 were successful and to also gel extract it for sequening
- Gel
  - 40 mL 1X TAE
  - 0.4 g of agarose
- DNA SAMPLES
  - 4 µL Loading Dye
  - 20 µL DNA
- 1kB Ladder
  - 6 µL 1 KB ladder (Truin Science)
- Stain for 20 mins
- De-stain for 30 mins
- Bands BARELY visible
- Stain for 20 mins
- De-stain for 30 mins

LANE	
1	J04500 dilution of maxiprep
2	K808000
3	K7-RFP miniprep
4	J04500 " "
5	K808000
6	K7-RFP miniprep

Gel Purification

- Date: June 19, 14
- Names: TDang

- Objective: After running our gel, we saw some good bands (1 and 5?)but 3 was really faint. Gel purified for DNA and for sequencing
- Instead of our usual protocol on page 59, we added Justin's tricks
- Procedure:
  1. Determine weight of gel, use weight before and after
  2. Add 4 times mg of Binding Buffer II. Heat at 60˚C for 10 mins
  3. Add to EZ-10 column, let stand for mins. Centrifuge at 4000 xg for 2 mins. Repeat to obtain all previous solution
  4. Add 750 µL wash solution, centrifuge for 10 000 rpm for 2 mins. Discard flow through
  5. Repeat step 4 2-3 times
  6. Centrifuge for an additional 2 mins to remove residual wash solution
  7. Transfer column to clean 1.5 mL microfuge tubes. Add 40 µL of MilliQ previously heated to 60˚C. Be sure to get as much of the water onto the filter as possible. Let stand for about 2 mins at 60˚C.
  8. Centrifuge at 12 000 rpm for 3 mins and store at 4˚C