Restriction of DNA(s)

- Date: May 1, 2014
- Names: Sunny, TDang, and Yoyo
- Overnight digestion in 37˚C incubator in E770

DNA	Restriction Sites
1X K331009-RFP-DT	X,P
1X J04500	S,P
1X J04500 – K331009	S,P
3X RFP-DT	X,P
1X K331008	S,P
1X K331007	S,P

Component	Volume (µL)
Milli-Q H2O	9
10 X Cutsmart Buffer	2
DNA	8
Enzyme 1	0.5
PST 1	0.5
Total	20

Nanodrops for May 1 Digestions

- Date: May 1, 2014
- Names: Zak

DNA	Concentration
J04500 MP 5/1	293 ng/µL
J04500 –K331009 5/1	364.3 ng/µL
K331007 (1)	360.3 ng/µL
K331007 (2)	407.2 ng/µL
K331007 (3)	411.8 ng/µL
K331008 (1)	321.5 ng/µL
K331008 (2)	379.5 ng/µL
K331008 (3)	315.1 ng/µL

Agarose Gel and Gel Extraction

- Date: May 2, 2014
- Names: Yoyo, Zak, Dinula, Ronja and Tdang
- After yesterday's ligations we will run a gel for parts RFP-DT and K331009-RFP-DT amd then (hopefully) extract the DNA *successfully?*
- 1 KB Ladder
  - 5 µL I KB Ladder
  - 1 µL Loading Dye
- Sample
  - 20 µL of DNA
  - 4 µL Loading Dye
- Run the gel for 25 mins @ 135 V
- Stained on EtBr for 20 mins
- Destained for 20 mins

Lane	
1	1 KB Ladder
2	K331009-RFP-DT
3	RFP-DT (1) (J04650)
4	RFP-DT (2)
5	The well is too close to the bottom of the mold to use
6	RFP-DT (3)

Gel Extraction

- Date: May 2, 2014
- Names: Yoyo, Zak, Dinula, Ronja, and TDang

	Tube	Initial Weight	Final Weight with Gel	Gel Weight	Amount of Binding Buffer II to add (multiply mass by 4)
K331009-RFP-DT	1	1.11 g	1.41 g	300 mg	1200 µL
RFP-DT (3)	2	1.11 g	1.44 g	330 mg	1320 µL
	RFP-DT			240 mg	960 µL

Gel Extraction Continued

- Date: May 2, 2014
- Names: Yoyo, Zak, Dinula, Ronja, and TDang

- **Protocol taken from page 42 of the lab book
- Cut gel with appropriate DNA fragment, transfer into a 1.5 mL microfuge tube
- Add the tube's respective amount of Binding Buffer II. Incubate at 60˚C for 10 mins, shaking occasionally
- Add Step 2 solution into EZ-10 columns. Add 800 µL increments to avoid overflow. Let stand for 2 mins, centrifuge at 10 000 rpm for 2 mins repeat until all solution is centrifuged. Discard the flow through
- Add 500 µL of wash solution, centrifuge at 10 000 rpm for 1 min. Discard solution in tube
- Place blue column in clean 1.5 mL microfuge tube. Add 40 µL of MilliQ water at 60˚C onto centre of the column. Incubate at 60˚C for 2 mins
- Centrifuge at 10 000 rpm for 2 mins. Discard EZ-10 columns and store purified DNA in -20˚C fridge

Ligations of K331008-RFP-DT, K331007-RFP-DT, J04500-K331009-RFP-DT, J04500-K331009-RFP-DT

- Date: May 2, 2014
- Names: Yoyo, TDang and Zak
- Zak's Ligation Rules
  - 3:1 ration for Insert:Vector
  - Total [DNA] ≤ 100 ng/mL
- To calculate vector concentration

$$\frac{[Miniprep]\ X\ Volume\ Added\ to\ Restriction}{Volume\ of\ Restriction}$$

- To calculate insert concentration

$$\frac{[Miniprep]\ X\ P\ X\ Volume\ Added\ to\ Restriction}{Elution\ Volume}$$

Ligations Continue

- Date: May 2, 2014
- Names: Yoyo, TDang and Zak

More Math

- For us today based on gel results
  - RFP-DT = 843 bp
  - pBICS = 2070 bp
  - Therefore 843 bp/2913 bp = 29%

  - K331009-RFP-DT = 900 bp
  - pMAT = about 2400 bp
  - Therefore 900/3300 bp = 27%

Ligations Continued

- Date: May 2, 2014
- Names: Yoyo, TDang and Zak

- **Check page 53 for concentrations of inserts
- Because vector concentrations are so high, dilute 10X to about the same concentration as insert

  C1V1=C2V2

  (140 ng/ µL)(V2) = (14 ng/ µL)(20 µL)

  V2 = 2 µL Therefore 2 µL of DNA is added to 18 µL of MilliQ Water for Vectors

Insert	Concentration	Vector Corresponding to Insert	Concentration
RFP-DT (Red 2)	11.5 ng/µL	K331008	140 ng/µL
RFP-DT (Red 2)	11.5 ng/µL	K331007	160 ng/µL
K331009-RFP-DT	18 ng/µL	J04500	117 ng/µL
RFP-DT (Black 1)	19.5 ng/µL	J04500-K331009	145.7 ng/µL

Ligation Continued

- Date: May 2, 2014
- Names: Yoyo, Tdang and Zak

Ligations (Similar to pg 37)	
Components	Volume (µL)
MilliQ Water	5
10 X Buffer (T4 Ligase Buffer)	2
Insert DNA	9
Vector DNA	3
Ligation Enzyme (T4 DNA Ligase)	1

Transformations of K331008-RFP-DT, K331007-RFP-DT J04500-K331009-RFP-DT and J04500-K331009-RFP-DT

- Date: May 3, 2014
- Names: Ronja, Sunny, TDang, Chris and Yoyo

- Thawed 20 µL of competent cells, from -80˚C freezer
- Gently pipette 1.8 µL of the DNA (K331008-RFP-DT, K331007-RFP-DT, J04500-K331009-RFP-DT, J04500-K331009-RFP-DT) into the corresponding (labeled) competent cells and pipet up and down a few times to ensure the cells and DNA mix
- Incubate the cells on ice for 30 mins
- Heat shock cells in 45˚C waterbath for 45 seconds
- Incubate cells on ice for 1 minute
- Add 250 µL pf sterile media to cells and incubate at 37˚C for 1 hour +15.5 mins
  - 3X SOC media used
  - 1X SOC media + TB media (not quite 250 µL of SOC media left)
- Plate cells and label LB plates on the outside perimeter

	Plasmid	Antibiotic Resistance
K331008	pMAT	AMP
K331007	pSB1AK3	AMP
J04500	pSB1AK3	AMP and KAN
J04500-K331009	pSB1C3	CAM

Picking Colonies

- Date: May 5, 2014
- Names: Dinula, Chris and Kieran

- Colonies Picked
  - K331008-RFP-DT AMP 1X
  - K331008-RFP-DT AMP 2X
  - K331008-RFP-DT AMP 3X

  - J04500-K331009-RFP-DT CAM 1X
  - J04500-K331009-RFP-DT CAM 2X
  - J04500-K331009-RFP-DT CAM 3X

  - K331009-RFP-DT KAN 1X
  - K331009-RFP-DT KAN 2X
  - K331009-RFP-DT KAN 3X

  - K331007-RFP-DT AMP 1X
  - K331007-RFP-DT AMP 2X
  - K331007-RFP-DT AMP 3X

- The K331009-RFP-DT KAN 1X, 2X and 3X did not grow and so were not included in the miniprep
- Placed in 37˚C RNA Common Room overnight.
- Also filled tips and tubes.

Miniprep of May 5 Colonies

- Date: May 6, 2014
- Names: Yoyo, Christina and Elaine

- **Cross-contamination of K331007-RFP-DT AMP 1X by K331007-RFP-DT 2X in 5 mL culture tubes
- Protocol of purification of plasmid DNA
- Added 2 mL overnight culture in tube, centrifuged @ 12 000 rpm
- As per miniprep protocol on pg 5 of iGem protocol book (BioBasic INC.)

Labeled Tube	DNA
A	K331008-RFP-DT AMP 1X
B	K331008-RFP-DT AMP 2X
C	K331008-RFP-DT AMP 3X
D	J04500-K331009-RFP-DT CAM 1X
E	J04500-K331009-RFP-DT CAM 2X
F	J04500-K331009-RFP-DT CAM 3X
G	K331007-RFP-DT AMP 1X
H	K331007-RFP-DT AMP 2X
I	K331007-RFP-DT AMP 3X

Transformation of J04500-K331009-RFP-DT

- Date: May 7, 2014
- Names: Yoyo and TDang

- Objective: Previous transformation of J04500-K331009-RFP-DT on KAN resistance plate was unsuccessful (moldy plates). Redoing transformation on AMP and KAN plates as the backbone plasmid should be pSB1AK3

- Thawed 20 µL of competent cells
- Added 1.8 µL of J04500-K331009-RFP-DT. Mixed with tip swirling
- Incubate cells on ice for 30 mins
- Heat shock cells at 42˚C for 45 sec
- Incubate cells on ice for 1 min
- Added 400 µL of LB media, incubated at 37˚C with shaking in RNA common room for 1 hour
- Plated cells on KAN and AMP resistence plates. 200 µL each in RNA commin room incubator at 37 ˚C

Digestion of May 6 Miniprep

- Date: May 7, 2014
- Names: Yoyo and TDang

- Objective: Miniprep concentration were low, somewhere around 30 ng/µL. Zak has sent them in for sequencing but while we wait, we willl digest at E and ? And run a gfel. This will confirm if our gene of interest is the appropriate size (around 800 bp)

- Incubate at 37˚C in E770 got 1 hour

Components of Digest	Volume (µL)
MilliQ Water	9
10X Cutsmart Buffer	2
1 to I minipreps from May 6	8
Pst 1 Enzyme	0.5
EcoR1	0.5

1% Gel of May 6 Minipreps

- Date: May 7, 2014
- Names: Yoyo and Tdang

- Note: Because we are running 9 samples of DNA we are using 2 ladders on either side of the minipreps
- Run gel in used 1X TAE for 30 mins on 135 V

1 KB Ladder	DNA
1 µL Loading Dye	20 µL DNA (A to I)
2 µL 1X TAE	4 µL loading dye
3 µL 1 KB Ladder	

1% Gel of May 6 Minipreps

- Date: May 7, 2014
- Names: Yoyo and TDang

Lane	
1	1 KB Ladder
2	K331008-RFP-DT AMP 1X (A)
3	K331008-RFP-DT AMP 2X (B)
4	K331008-RFP-DT AMP 3X (C)
5	J04500-K331009-RFP-DT CAM 1X (D)
6	J04500-K331009-RFP-DT CAM 2X (E)
7	J04500-K331009-RFP-DT CAM 3X (F)
8	K331007-RFP-DT AMP 1X (G)
9	K331007-RFP-DT AMP 2X (H)
10	K331007-RFP-DT AMP 3X (I)
11	1 KB Ladder

Picking Colonies

- Date: May 7, 2014
- Names: Yoyo and TDang

- Objective: Pick colonies from previous ligations. Pick only colonies with successful ligations, based on gel results

- Worked in sterile environment under open flame
- Incubated in 37°C shaker overnight in RNA Common room

Picked Colonies
K331008-RFP-DT AMP 1X
J04500-K331009-RFP-DT CAM 2X
J04500-K331009-RFP-DT CAM 3X
K331007-RFP-DT AMP 3X

Miniprep of K331008-RFP-DT AMP 1X (A), K331007-RFP-DT AMP 3X (I), J04500-K331009-RFP-DT CAM 2X (E) and J04500-K331009-RFP-DT AMP 3X (F)

- Date: May 8, 2014
- Names: Yoyo and Ronja

- The same procedure was followed as the miniprep protocl on pg 29
- Purified DNA stored in -20°C fridge in compartments D3 to D6

Picking Colonies from May 7 Transformations

- Date: May 8, 2014
- Names: Yoyo and Ronja

- Sterile environment under open flame!
- Two plates, both with J04500-K331009-RFP-DT one on KAN and the other on AMP

- Picked three colonies per plate. Tubes labelled with:
  J04500-K331009-RFP-DT pSB1AK3
  KAN or AMP with Pick Site number
  HS iGem Y.Y. R.K.
  May 8, 2014

- Tubes left in RNA Common Room in 37°C shaker to grow overnight

Sequencing Results

- Date: May 9, 2014

- K331007-RFP-DT → Good!
- K331008-RFP-DT → Bad!
- K331009-RFP-DT → Crazy bad
- K331009 alone → Only red is good marker
- J04500 _K331009-RFP-DT → No K9 … bad …

- Lessons learned
  - Fully digest plasmids
  - Sequence at every step

Digest of J04500-K331009-RFP-DT CAM 2X from May 6, 2014

- Date: May 9, 2014
- Names: Ronja, Dinula, Yoyo and Zak

- Incbuate J04500-K331009-RFP-DT for 1 hour in room E770
- Note: J04500-K331009-RFP-DT from May 6 is missing signal sequence

Components	Volume (µL)
MilliQ Water	7
10X Cutsmart Buffer	2
pDNA (J04500-K331009-RFP-DT from May 6)	10
EcoR 1	1
PST 1	1

Restriction of May 6 K331008

- Date: May 8, 2014
- Names: Ronja, Dinula, Yoyo and Zak

- Objective: We do not know of ligations worked for J04500-K331009 (F). We are going to compare the May 8 bands to the May 6 bands to see if the ligation worked. The May 6 J04500-K331009 we know from sequencing that if it does not have a signal sequence.
- Overnight digest in 37°C incubator in E770.

Restriction of May 6 K331008	
Components	Volume (µL)
MilliQ Water	8.6
10X Cutsmart Buffer	2
DNA	8
Spec 1	0.7
PST 1	0.7

2.5% Agarose Gel

- Date: May 9, 2014
- Names: Yoyo, Dinula and Ronja

- Objective: We are making a 2.5% agarose gel with our 1 hr restrictions (J04500-K331009-RFP-DT CAM 2X from May 8, J04500-K331009-RFP-DT CAM 3X from May 8 and J04500-K331009-RFP-DT from May 6) in order to test if our ligations worked from May 2
- For the agarose gel
  - 20 mL 1X TAE
  - 0.5 g Agarose
- 6.5 µL of ladder, 25 µL in Lane 2, 25 µL in Lane 3 and 25 µL in Lane 4 were added
- Note for Lanes 2-4 20 µL of plasmid was added with 4 µL of loading dye
- Running at 50 V for 99 mins

Lane	
1	Ladder • 3 µL of KB Ladder • 2 µL of 1X TAE • 1 µL Loading Dye
2	J04500 –K331009-RFP-DT CAM 2X (Cut E, P on May 8)
3	J04500 –K331009-RFP-DT CAM 2X (Cut E, P on May 8)
4	J04500 –K331009-RFP-DT CAM 3X (Cut E, P on May 6)

Overnight Restriction

- Date: May 9, 2014
- Names: Yoyo, Ronja and Dinula

- Objective: Start assembly on the K331007-RFP-DT plamid with promoter J04500
- K331007-RFP-DT Cut X and P
- 2 X J04500 Cut S and P

Components	Volume (µL)
MilliQ Water	8.6
10X Cutsmart Buffer	2
pDNA	8
Enzyme 1	0.7
Enzyme 2	0.7

Picking Glycerol Stocks

- Date: May 9, 2014
- Names: Yoyo, Zak, Ronja and Dinula

- Objective: Pick new stocks of cells with out DNA to work with. Our sequencing came back with interesting results, this, we want to start fresh with new DNA
- In 37°C shaker in RNA Common Room. Left to grow overnight

Picked Colonies	
K331009 in AMP	5 mL LB Tubes X 4
K331008 in AMP	5 mL LB Tubes X 4
RFP-DT in CAM	5 mL LB Tubes X 4

Re-Grow May 8 Picked Colonies

- Date: May 9, 2014
- Names: Yoyo, Zak, Ronja and Dinula

- Objective: We left the May 8 colonies in 5 mL LB media tubes in the shaker for 24 hours … opps! Because the AMP antibiotics could be degraded in that time, allowing other bacteria to grow we will re-grow them with new LB media tubes with antibiotics.
- Labelled tubes with:

  J04500-K331009-RFP-DT pSB1AK3

  KAN or AMP with Pick Site number (1 to 3)

  HS iGem

  May 9, 2014

Miniprep of May 9 Stocks

- Date: May 10, 2014
- Names: Ronja and Yoyo

- Objective: Miniprep to obtain purified DNA from glycerol stocks picked last night. As well obtain DNA of J04500-K331009-RFP-Dt from AMP and Kan picked colonies
- Followed protocol from page 29 of lab book
- Plasmids miniprepped
  - K331009 in AMP 4X
  - K331008 in AMP 4X
  - RFP-DT in CAM 4X
  - J04500-K331009-RFP-DT in KAN 3X
  - J04500-K331009-RFP-DT in AMO 3X

1% Agarose Gel and Gel Extraction

- Date: May 10, 2014
- Names: Ronja Kothe, Yoyo Yao

- Objective: To extract and purify our insert DNA K331007+RFP-DT, for future ligations.
- Gel
  - 20µL 1x TAE
  - 0.2g Agarose
- Ladder Components
  - 3µL 1kb Ladder
  - 2µL 1x TAE
  - 1µL 6x Loading Dye
- K7 Components
  - 20µL Restriction DNA (heat killed enzymes)
  - 4µL 6x Loading Dye
- *K7 well was short of 24µL
- Gel ran for 135V at 25 minutes
- Stained in EtBr for 20 mins
- Destained for 20 mins

Lane	
1	1kb Ladder
2	K331007+RFP-DT

Gel Extraction

- Date: May 10, 2014
- Names: Ronja and Yoyo

- Weight Before: 1.13 g
- After:
- Never mind … no bands can be sedn on our second lane. There's dye visible, but no bands. Cry …

Digestion Overnight

- Date: May 11, 2014
- Names: Yoyo

- Objective: Previous restriction of K331007-RFP-DT didn't seem to work. Re-doing that restriction for future ligations.
- The volumes were repeated for 2 tubes.
- Left in E770 at 37˚C incubator overnight. In @ 5:55 pm

Component	Volume (µL)
Milli-Q H2O	8.6
10 X Cutsmart Buffer	2
K331007-RFP-DT	8
Xba1	0.7
PST 1	0.7

1% Agarose A made with 1X TAE (Ran in used 1X TAE)

- Date: May 12, 2014
- Names: Yoyo and Wes

- Objective: Extraction of insert plasmid of K331007-RFP-DT
- For the Ladder
  - 1 µL Dye
  - 2 µL of 1X TAE
  - 3 µL of 1 KB Ladder
- K331007-RFP-DT (1 and 2)
  - 20 µL DNA
  - 4 µL Loading Dye
- Run for 30 mins at 135 V
- Stained in EtBr for 20 mins
- Destained for 20 mins
- Looks like there are no bands visible in lanes 2 and 3. Ladder barely showed up … This is the second time this has happened, I'm wondering how much/if any DNA is in our K331007-RFP-DT

Lane	
1	1 KB Ladder
2	1 (K331007-RFP-DT)
3	2 (K331007-RFP-DT)

Digest of RFP-DT (X2), K8 and K9

- Date: May 12, 2014
- Names: Yoyo and Wes

- Objective: Overnight digest of glycerol stock of RFP-DT (X, P) K331008 (S, P) and K331009 (S, P). For future ligations/ assemblies.
- Overnight digest at 37˚C in E770. In at 7:50 pm

Component	Volume (µL)
Milli-Q H2O	8.6
10 X Cutsmart Buffer	2
K331007-RFP-DT	8
Enzyme 1	0.7
Enzyme 2	0.7

DNA	Enzymes
2 X RFP-DT	X, P
K331008 (Used Tube #1)	S, P
K331009 (Used Tube #1)	S, P

Running Gel of PCR Parts and Restriction of J04500

- Date: May 13, 2014
- Names: Chris

- Restriction was left to incubate at 37˚C in E770 overnight.

Component	Volume (µL)
Milli-Q H2O	9
10 X Cutsmart Buffer	2
K331007-RFP-DT	8
Spec1	0.5
Pst1	0.5
Total	20

Gel and Gel Extraction

- Date: May 12, 2014
- Names: Chris

- PCR components were run on 1X TAE agarose gel at 135 V for 30 mins
- The gel was successful!
- The PCR products were consolidated and rotovapped for 10min to concentrate
- Restriction was left in E770.

Component	Volume (µL)
Milli-Q H2O	9
Cut smart	2
DNA	8
Xbal1	0.5
Pst1	0.5

Ligation of K331007 with J04500

- Date: May 14 2014
- Names: No name

- Objective: Ligase signal sequence to promoter vector

- 3 part insert to 1 part vector
- Cut site x and p for K331007, cut site s and p for J04500
- Concentrations: K331007-350ng/µL, J04500-15ng/µL
- This is not 3:1 due to special effects from PCR, ask an advisor for details.

Component	Volume (µL)
Milli-Q H2O	4
10 X Cutsmart Buffer	2
K331007	2
J04500	10
Enzyme	2
Total	20

Picking Colonies

- Date: May 14, 2014
- Names: Yoyo

- Objective: Obtain more K331007 DNA for future ligations

- Aseptic environment, open flame
- Added 5µL of AMP to two tubes of 5ml LB media
- Picked colonies: #1 and #2 (pick site)
- Tubes labeled: K31007 pMAT - AMP 1 or 2 – May 14,14 – Y.Y.
- Tubes in RNA common room shaker. Overnight for 37°C with shaking

Transformation of J04500-K331007-RFP-DT

- Date: May 15, 2014
- Names: Christina, TDang, Chris and Zak

- Continuing the process of the project and transform the successful ligation of J04500-K331007-RFP-DT
- Following the same procedure as pg 38 for the transformation. However we did a transformation so 1.8µL of the ligation to the tube of the competent cells
- The transformations were divided by a (half of one tube in CAM and other half in AMP (200µL))

- Tube 1 of transformation:
  - AMP – (J04500-K331007-RFP+DT) 1
  - CAM – " " 1

- Tube 2 of transformation:
  - AMP – " " 2
  - CAM – " " 2

- Incubate overnight at 37 degrees in RNA common room

Picking Glycerol Stocks

- Date: May 15, 2014
- Names: Christina, Tdang, Chris and Zak

- 6 tubes of 5µL LB media labeled with either K331008, K331009, RFP-DT
- Stocks were picked from glycerol stocks from the -80 degrees freezer in E770. Worked aseptically under open flame.
- Tubes labeled K331008 (1 and 2), K331009 (1 and 2), RFP-DT (1 and 2)
- Tubes in RNA common room shaker. Overnight at 37 degrees with shaking.

Miniprep of May 15th glycerol stocks and K331007-RFP-DT colonies from May 14th

- Date: May 16th 2014
- Names: Chris, TDang and Ronja

- Objective: to isolate more DNA from future testing

- Protocol on pg 29 followed
- Stored in HS iGEM -20°C degrees box #1

Digests of RFP-DT,K9 and K8

- Date: May 16 2014
- Names: Wes, Dinula and TDang

- Objective: Prepare DNA for ligation (gel confirmation first)

- Concentration of minipreps in chart above
- Restriction based on pg 38
- For RFP-DT used Xbal and Pst1 (Insert)
- For K8/K9 used Spel and Pst1 (Vector)
- Digest overnight at 37°C

Component	Concentration (ng/µL)
K7 – R (1)	198
K7 – R (2)	238
RFP-DT (1)	116
RFP-DT (2)	153.4
K8 (1)	335.6
K8 (2)	192
K9 (1)	190
K9 (2)	189.6

Component	Volume (µL)
Milli-Q H2O	7
10 X Cutsmart Buffer	2
DNA (K8,K9,RFP-DT)	10
Enzyme 1	0.5
Enzyme 2	0.5

Gel of J04500-K331007-RFP+DT

- Date: May 16 2014
- Names: Wes, Dinula, and TDang

- The transformations from May 15,2014 were checked the next day but no visible colonies grew. Zak ran a PCR of the transformations and will run a 2% agarose gel to make sure the ligations worked.
- For Agarose Gel
  - 0.8g of agarose gel
  - 40ml TAE 1x

Digest

- Date: May 19,2014
- Names: No name

- Objective: Cut stock parts of K8,K9 and K7-RFP+DT, RFP-DT for future assembly

- K9 and K8 cut with S and P
- RFP-DT and K7-RFP-DT cut with X and P
- Left in red thermocycler in E770 for 4hrs at 37°C. Pre-programmed cycle used.

Component	Volume (µL)
Milli-Q H2O	6
10 X Cutsmart Buffer	2
DNA (K8,K9,RFP-DT)	10
Enzyme 1	1
Enzyme 2	1

Component	Volume (µL)
Milli-Q H2O	11
10 X Cutsmart Buffer	2
DNA (K7+RFP-DT)	5 (only 5 because we want to save some for transformation)
Enzyme 1	1
Enzyme 2	1

Transformation of K331007-RFP+DT Miniprep

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

- The May 15th transformations were unsuccessful as no colonies grew. Zak said to transform the successfully sequenced K331007-RFP+DT and will miniprep.

- Following the protocol on pg 38
- Grab 3 20µL of competent cells from -80 degrees Celsius freezer and thaw
- Pipette 1 µL of DNA into each tube into the competent cells and pipette up and down to rinse the tip
- Incubate the cells on ice for 30mins
- Heat shock the cells in water bath at 42 degrees Celsius for exactly 45 secs
- Add 250 µL of sterile media to the cells and incubate at 37 degrees Celsius for 1hr with shaking in the RNA common room
- Plate transformation on AMP plates stolen from Harland

- Transformation of May 10th Minipreps:
- Tube G: K331007-RFP+DT
- Tube H: K331007-RFP+DT
- Tube I: K331007-RFP+DT

Picking Glycerol Stocks

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

- Picking glycerol stocks of J04500 and J04650 (RFP) to miniprep and provide for more needed DNA. Glycerol stocks are placed in 5ml LB media tube

- 1x J04500 with 5 µL KAN
- 3x J04650 with 5 µL CAM

Gel Extraction

- Date: May 21, 2014
- Names: Chris

Lane	Content
1	1KB Ladder
2	K331007-RFP-DT (X,P)
3	RFP-DT (X,P)
4	K331008 (S,P)
5	K331009 (S,P)

Content	Volume (µL)
K331007-RFP-DT	160 of binding buffer
RFP-DT	160 of binding buffer
K331008	160 of binding buffer
K331009	200 of binding buffer

Gel Purification

- Date: May 21, 2014
- Names: TDang and Yoyo
- Followed pg 59 gel purification protocol, adjusting to gel weight

Ligations of K331008/K331009

- Date: May 21, 2014
- Names: Tdang and Yoyo

- Objective: Assembly of K331008/ K331009 with RFP-DT
- ** Since our concentration of vector and insert are curretnly unknown, we will try three different ligations for our two parts (K8/K9). Tomorrow Graeme will test concentrations with nanodrops for us.
- Overnight at 16˚C

Component	Volume (µL)
Milli-Q H2O	*9, 7, 5
T4 Ligase Buffer	2
DNA Vector (K331008/ K331009)	4
DNA Insert (RFP-DT)	*4, 6, 8
Ligase Enzyme	1

Minipreps of May 19 Glycerol Stocks

- Date: May 21, 2014
- Names: TDang and Yoyo

- Seems we are running low on RFP-DT and J04500, so we miniprepped the May 19 glycerol stocks which were picked.
- J04500 (pSB1AK3) and 3X for RFP-DT
- Same procedure followed as page 29 of the lab book

- Tubes labeled

  J04500 (pSB1AK3)
  May 21, 14
  T.D. Y.Y.

  Or

  RFP-DT (1X or 2X or 3X)
  May 21, 14
  T.D. Y.Y.

Nanodrop Concentrations

- Date: May 21, 2014
- Names: Graeme

- Objective: Graeme will nanodrop stuff for us to find concentrations! Good for ligations and such
- **Note: Negative concentrations most likley due to buffer solution left over from restrictions. Graeme double checked #3 for us, replacing his nanodrop test with buffer rather than water, and got a more reasonable number.
- 5 µL volume for nanodrops

Tube Number	DNA	Date	Concentration (ng/µL)
1	K331009 (Cut @ S, P)	May 21, 2014	-9.0
2	K331008 (Cut @ S, P)	May 21, 2014	-4.2
3	K331007-RFP-DT (Cut @ X, P)	May 21, 2014	47.1
4	RFP-DT (Cut @ X, P)	May 21, 2014	-7.5
5	J04500 pSB1AK3	May 21, 2014	534.8
6	RFP-DT 1X	May 21, 2014	280.2
7	RFP-DT 2X	May 21, 2014	283.8
8	RFP-DT 3X	May 21, 2014	256.6

Transformation

- Date: May 22, 2014
- Names: Yoyo and Tdang

- Objective: Transform yesterday's ligations of K8-RFP-DT (X3) and K9-RFP-DT (X3). Eventually do a colony PCR to confirm if ligations were successful.

- Followed protocol on page 38 of the lab book.
- All on AMP plates!
- In 37˚C incubator in RNA Common Room for 0/N

Parts Transformed	
K331008+RFP-DT	4 µL DNA
" "	6 µL DNA
" "	8 µL DNA
K331009+RFP-DT	4 µL DNA
" "	6 µL DNA
" "	8 µL DNA

Digest of J04500

- Date: May 22, 2014
- Names: Yoyo and TDang

- Objective: Restrict J04500 at S and P for future assembly with K7+RFP-DT

- ** J04500 in smaller quantity then usual as it is so concentrated
- Left 0/N in 37˚C incubator in E770.
- Heat killed restriction enzymes with 20 mins @ 65˚C via thermocycler.

Component	Volume (µL)
Milli-Q H2O	13
10 X Cutsmart Buffer	2
J04500 pSB1AK3	3
Enzyme 1	0.5
Enzyme 2	0.5

Ligation of J04500+K7+RFP-DT

- Date: May 23, 2014
- Names: Yoyo

- Ligation tube left in D770 drawer for 2 nights due to Calgary workshop

Components	Volume (µL)
MilliQ Water	8
10 X Buffer (T4 Ligase Buffer)	2
DNA Vector (J04500)	4 (After 1/40 dilution)
DNA Insert (K7-RFP-DT)	5
Ligation Enzyme (T4 DNA Ligase)	1

Ligation of J04500+K7+RFP-DT

- Date: May 23, 2014
- Names: Yoyo

- Concentration of Insert (K7+RFP-DT) = 47. 1 ng/µL
- Concentration of Vector (J04500) = 534.8 ng/µL
- Need 3:1 Ratio of Insert to Vector (Concentration)
- Need ≤ 100 ng/µL of DNA Total
- Vector Concentration = $\frac{[MP]\ X\ Volume\ added\ to\ Restriction}{Volume\ Restriction}$

  = 80.22 ng/ µL (Need to dilute 1/40)

- Insert Concentration = $\frac{[MP]\ X\ \%\ X\ Volume\ added\ to\ Restriction}{Volume\ Restriction}$

  = 4.98 ng/µL

- Note: K7+RFP-DT = 843 + 69 bp and pSB1A3 = 2155 bp so the % = 42.3%
- 4 µL of Vector (J04500) = 8 ng/ µL after 1/40 dilution
- 5 µL of Insert (K331007+RFP-DT) = 24 ng/µL
- 1:3 ratio achieved

Transformation of J04500+K7+RFP-DT

- Date: May 26, 2014
- Names: Yoyo

- Objective: Transform our J+K7+RFP-DT, create colonies to do PCR's with to confirm ligations
- Followed protocol on page 38

J04500+K331007+RFP-DT

- Step 7) Added 500 µL of LB Media
- Step 8) Added 250 µL of cells onto AMP plate, the other 250 µL of cells onto KAN

PCR of K8-RFP-DT and K9-RFP-DT Ligations

- Date: May 27, 2014
- Names: Yoyo, Wes, Kieran, Christina, Sunny, Tiffany and TDang

Protocol for PCR

- Using a marker, label the colonies that will be isolating template form
- Correspondingly label as many thin walled PCR tubes as necessary (For us 18 tubes)
- Add 20 µL of Milli-Q Water into the labeled PCR tubes
- Touch a sterile tip to the colony of interest (for a list of colony of interest refer to the next slide)
- Place the end of the tip into the corresponding tube and transfer the cells by pipeting up and down or stirring with the tip
- Briefly vortex the tubes
- Centrifuge the tubes @ 10 000 rpm for 2 minutes to spin down cell debris
- After centrifugation, the samples are suitable for use as template. Add 1 µL of DNA in PCR reaction
- After add 24 µL of Master Mix into each of the 18 tubes and then add 1 µL of respected DNA into the respected tubes
- ** Before adding the master mix to the sample tubes, give it a slow vortex and spin down
- Leave in the thermocylcer for 3 hours however because none of us want to be here until midnight the NDA tubes will be left at 4˚C in the thermocycler until the morning when someone can run it
- ** Note: However for some strange reason we ran out of the Master Mix so we made a second Master Mix in which we multiplied the volume by 4

Master Mix for 18 Samples of Ligations (Volume X 18)	
Components	Final Volume (µL)
Milli Q Water	266.4
10 X Biobasic TAQ Reaction Buffer	45
10 mM dNTPs	9
10 mM Biobasic $MgCl_2$	36
10 µM primer 1	36
10 µM primer 2	36
5 µ/µL Biobasic TAQ Polymerase	3.6

Master Mix (Volume X 4)	
Components	Final Volume (µL)
Milli Q Water	59.2
10 X Biobasic TAQ Reaction Buffer	4510
10 mM dNTPs	2
10 mM Biobasic $MgCl_2$	8
10 µM primer 1	8
10 µM primer 2	8
5 µ/µL Biobasic TAQ Polymerase	0.8

PCR of K8-RFP-DT and K9-RFP-DT Ligations

Picking Colonies			
Tube Number	DNA	Volume of Insert in Ligation (µL)	Colony Number
1	K331009+RFP-DT	8	Colony 1
2	K331009+RFP-DT	8	Colony 2
3	K331009+RFP-DT	8	Colony 3
4	K331009+RFP-DT	4	Colony 1
5	K331009+RFP-DT	4	Colony 2
6	K331009+RFP-DT	4	Colony 3
7	K331008+RFP-DT	4	Colony 1 (The only one)
10	K331008+RFP-DT	8	Colony 1
11	K331008+RFP-DT	8	Colony 2
12	K331008+RFP-DT	8	Colony 3
13	K331008+RFP-DT	6	Colony 1
14	K331008+RFP-DT	6	Colony 2
15	K331008+RFP-DT	6	Colony 3
16	K331009+RFP-DT	6	Colony 1
17	K331009+RFP-DT	6	Colony 2
18	K331009+RFP-DT	6	Colony 3

1% Agarose Gel

- Date: May 28, 2014
- Names: Yoyo

- Objective: Run our PCR'd DNA on an agarose gel, see if our ligations were successful. Successful bands should be about 1000 bp

- For the Ladder
  - 10 µL 1 KB Ladder (pre mixed)
- DNA X 16
  - 10 µL DNA
  - 2 µL Loading Dye
- Tubes 7, 11 and 17 low in PCR Tubes
- Pan gel for 45 mins at 125 V

Lane	
1	1 KB Ladder
2	# 1
3	# 2
4	# 3
5	# 4
6	# 5
7	# 6
8	# 7
9	# 10
10	# 11
11	# 12
12	# 13
13	# 14
14	# 15
15	# 16
16	# 17
17	# 18
18	1 KB Ladder

Ligations of J04500-K331007-RFP-DT

- Date: May 28, 2014
- Names: Tiffany, TDang and Rachel

- Objective: We had interesting results from the gel but no bands at about 1000 bp. Yoyo is going to talk to Zak and/or Justin about our findings. In the meantime a ligation of j04500-K331007-RFP-DT will be done tonight as no colonies grew on our plate and we lost the previous ligated DNA

- Both tubes incubated at 37˚C overnight left in the drawer where notebook is usually stored

Component	Volume (µL)
Milli-Q H2O	8
T4 Ligase Buffer	2
DNA Vector (J04500)	4 (after 1/40 dilution)
DNA Insert (K7-RFP-DT)	5
Ligase Enzyme	1

Component	Volume (µL)
Milli-Q H2O	8
T4 Ligase Buffer	2
DNA Vector (J04500)	3 (after 1/40 dilution)
DNA Insert (K7-RFP-DT)	6
Ligase Enzyme	1

Picking Colonies

- Date: May 29, 2014
- Names: Yoyo and Sunny

- Picked from competent cells with no antibiotic resistance into two beakers of 50 mL LB Media
- Tubes in 4˚C fridge for now
- Left in 37˚C shaker in RNA Common Room for overnight

Digestion

- Date: May 29, 2014
- Names: Sunny and Yoyo

- Objective: Cut stock parts of K9 and K8, RFP-DT for future assembly
- K331009 and K331008 cut with S and P
- RFP-DT cut with X and P

Component	Volume (µL)
Milli-Q H2O	6
10 X Cutsmart Buffer	2
DNA	11
Enzyme 1	0.5
Enzyme 2	0.5
Total	20

UV Spectrophotometer –Determining DNA Concentration

- Date: May 29, 2014
- Names: Sunny, TDang and Yoyo

- Objective: Determine the concentrations of the 10 tubes of DNA

- The chart is on the following slide

UV Spectrophotometer –Determining DNA Concentration

Tube #	DNA	260 nm	280 nm	Concentration (ng/µL)
1	K331008-RFP-DT (May 8, 2014)	0.014	0.000	35.7
2	K331007-RFP-DT (May 8, 2014)	0.023	0.012	58.65
3	J04500 –K331009 –E8 (May 8, 2014)	0.031	0.016	79.05
4	J04500 –K331009 –F (May 8, 2014)	0.029	0.017	73.95
5	J04500 -K331009-RFP-DT (May 10, 2014) (KAN 1)	0.019	0.012	48.45
6	J04500 -K331009-RFP-DT (May 10, 2014) (KAN 2)	0.039	0.031	99.45
7	J04500 -K331009-RFP-DT (May 10, 2014) (KAN 3)	0.020	0.016	51.00
8	J04500 -K331009-RFP-DT (May 10, 2014) (AMP 1)	0.043	0.037	109.65
9	J04500 -K331009-RFP-DT (May 10, 2014) (AMP 2)	0.019	0.014	48.45
10	J04500 -K331009-RFP-DT (May 10, 2014) (AMP 3)	0.027	0.020	68.85

Sending May 29, 2014 UV Spec DNA for Sequencing

- Date: May 29, 2014
- Names: Sunny, TDang and Yoyo

Primer Dilutions for Sequencing

First Dilution
V = 5 µL of 10 mM + 45 µL MilliQ Water

Second Dilution (10 mM to 25 pM)
V = 2.5 µL of 1 mM + 47.5 µL MilliQ Water

Third Dilution
V = 6 µL of 50 µM + 54 µL MilliQ Water
5 µL of 25 pM into each tube

Notes
- VR –Black tubes/marker
- VF2 –Red tubes/marker

Making DH5α Competent Cells

- Date: May 30, 2014
- Names: TDang and Yoyo

- Objective: After the results from the gel we need to plan what to do next. 10 samples of DNA (on the previous page) have been sent out for sequencing on May 28, 2014. In the meantime we made more DH5α competent cells as we are running low on them for transformations … 60 tubes of them

1. Centrifuge 50 mL (X2) culture at 2700 XG for 7 mins at 4˚C
2. Pour off supernatant being careful not to disturb the pellet. Tap gently on paper towel to remove any remaining supernatant.
3. Add 15 mL ice cold 80 mM $MgCl_2$, 20 mM $MgCl_2$ to the tube. Resuspend by pipeting up and down gently (do not vortex)
4. Centrifuge for 5 mins at 2700 XG
5. Pour off supernatant
6. Resuspend cell pellet in 2 mL ice cold 100 mM $CaCl_2$
7. Add 0.6 mL sterile glycerol and mix gently
8. Aliquate samples (20 µL), flash freeze with liquid nitrogen and store at -80˚C

Transformation of J04500-K331007-RFP-DT

- Date: May 31, 2014
- Names: Yoyo and Tdang

- Objective: Transformation of J-K7-RFP-DT (X2 as there are two ligations from May 28, 2014) onto 2 plates of AMP and KAN for future PCR to determine if the ligations were successful.
- ** Each ligation of J04500-K331007-RFP-DT was plated on AMP and KAN (plasmid pSB1AK3)
- **Protocol followed on page 38-39 of the lab book

1. 4 20 µL of competent cells grabbed from -80˚C freezer to thaw
2. Pipet 1.8 µL of DNA into each tube of competent cells and pipet up and down once to rinse the tip
3. Mix the DNA into the cells by swirling the tip in the solution
4. Incubate the cells on ice for 30 mins
5. Heat shock the cells in water bath at 42˚C for exactly 45 seconds. Leave on ice for 1 min after
6. Add 400 µL of sterile LB media to the cells and incubate at 37˚C for 1 hour with shaking
7. Label 2 AMP plates and 2 KAN plates
8. Plate each tube onto respected plate and let place soak in the suspension
9. Fkip the plate over and incubate in the 37˚C oven in the RNA Common Room

Picking Colonies

- Date: May 31 2014
- Names: TDang, Yoyo

- Objective: Picking colonies of K7+RFP-DT, increase our stock amount of DNA just in case.

- Pick sites are A and B, two plates labeled I and H.
- Work sterile! Open flame!
- Picked with a P2, dropped pipette tip into 5mL of sterile LB media.
- Left in RNA common room to grow overnight. 37 degrees Celsius with shaking.