Ligation of RFP-DT and K331009

- Date: April 1, 2014
- Names: Dinula and Chris

Band	Area	Concentration
1	2383.355	70µg/µL
2	1695.284	25µg/µL
3	2052.577	25µg/µL
4	4042.305	X µg/µL

Ligation of BBa_K331009 and RFP-DT

- Date: April 1, 2014
- Names: TDang

- Incubate at room temperature overnight

Components	Volume (µL)
Milli-Q H2O	4
10x Buffer (T4 Ligase)	1
DNA $_1$ (RFP)	4
DNA $_2$ (K331009)	0.5
Ligation Enzyme	0.5
Total	10

Transformation

- Date: April 2, 2014
- Names: TDang

- Procedure
  1. Heat Kill Ligase → 65˚C for 10 min
     → Centrifuge for 2 min at 10, 000 rpm
  2. Remove competent cells from - 80˚C freezer and place on ice.
  3. Put 10% (V/V) Ligation into competent cell mix.
  4. Flick **gently** ~ 5 times to mix.
  5. Incubate on ice for 15 minutes.
  6. Heat shock cells for 30 seconds at 42˚C
  7. Place cells on ice for 5 minutes.
  8. Aseptically add 250 µL room temperature soc media to cell mix.
  9. Incubate while shaking (220 rpm) at 37˚C for ~ 1 hour (7:50 pm)
  10. Plate cells pre-warmed LB media with agar plates containing the appropriate antibiotic .
  11. Incubate overnight at 37˚C (remember agar up)

Growing and Picking our Colonies

- Date: April 3, 2014
- Names: Ronja and TDang

- Procedure:
  1. Get 10x 5 ml LB tubes.
  2. Get appropriate antibiotic.
  3. Get plates from incubator.
  4. Add antibiotic to tubes aseptically (5µL)
  5. Pick and mark 10 colonies
     a) scoop with pipette and add to tube
  6. Label everything!
  7. ***incubated in RNA common room at 37˚C overnight

Materials
7x 5ml LB Medias
5 µL AMP antibiotic in each
5 colonies of NEBa pHA-T BBa_K331009 + RFP-DT
2 colonies of NEBa pSBIAT3

Miniprep—Plasmid DNA

- Date: April 4, 2014
- Names: Yoyo and Kieran

- Procedure
  1. Placed 1.5ml of cultured cells into respective tubes and labeled
  2. Centrifuged at 1200rpm for 2 minutes
  3. Disposed of supernatant
  4. Added 100 µl of solution 1 and mixed with pellet
  5. Added 200 µl of solution 2 and inverted 6 times each
  6. Added 350 µl of solution 3 and gently mixed
  7. Centrifuged at 12 000rpm for 5 minutes
  8. Transferred supernatant to the EZ-16 column (labeled)
  9. Centrifuged at 10 000rpm for 2 minutes (discarded supernatant)
  10. Added wash solutions and centrifuged at 10 000rpm for 2 minutes, two times
  11. Discarded flow-through, spun for 1 minute
  12. Discarded flow-through tubes
  13. Placed EZ-10 columns into 1.5 microfuge tubes
  14. Added 50 µl elution buffer, spun for 10 000rpm for 2 minutes
  15. Purified DNA in -20˚C fridge

Digestion of Today's Miniprep

- Date: April 4,2014
- Names: Blank

- Into 37˚ incubator at 2:45pm

Test Tube	
1	K331009+RFP+DT
2	" + "
3	" + "
4	" + "
5	pSBIAT3

Components	Volume (µL)
MILLI-Q H2O	5.5
10 Cutsmart Buffer	1
pDNA	3
EcoR1	0.5
Total	10

Running an Agarose Gel to Test Ligation

- Date: April 4, 2014
- Names: TDang and Ronja

- Procedure:
  1. Make 1% gel solution (1g agarose, 100ml TAE)
  2. Heat to dissolve solute
  3. Let cool (warm shower temperature) then pour into the gel cast (with comb)
  4. Led solidify (15mins ±5mins.)
  5. Load gel (loader and samples)
  6. Run for 40mins. At 100V in TAE buffer 10x diluted 1x

Lane	5µl DNA + 1 µl Loading Dye = 6µl
1	1kb ladder (6µl)
2	Purified (6µl)
3	Sample1 (6µl)
4	Sample2 (6µl)
5	Sample3 (6µl)
6	Sample4 (6µl)
7	Sample5 (6µl)

Miniprep of PSBIAT3-RFP-DT

- Date: April 5, 2014
- Names: Sunny, TDang and Logan

- Procedure:
  1. Took 2ml of cultured cells into tubes.
  2. Centrifuged at 12,000 rpm for 2 mins.
  3. Discarded supernatant into waste beaker.
  4. Added 100µl of solution 1 and resuspended.
  5. Added 200µl of solution 2 and inverted.
  6. Added 350 µL of solution 3 and mixed gently.
  7. Centrifuge at 10 000 rpm for 2 mins.
  8. Discard flow through.
  9. Added wash solution (750 µL) and centrifuged at 10 000 rpm for 2 minutes –twice
  10. Discarded flow through and centrifuge column empty for 1 min.
  11. Discarded flow through tubes.
  12. Placed Ez-10 columns into labeled 1.5 microfuge tubes.
  13. Added 50 µL elution buffer and spun for 2 mins at 10 000 rpm.

Loading and Running Agarose Gel

- Date: April 5, 2014
- Names: Sunny, Logan and Tdang

- Lineralized DNA (Restriction)
- DNA SAMPLES
  - 2 µL Loading Dye
  - 10 µL DNA
- 1kB Ladder
  - 1 µL Loading dye
  - 2 µL TAE Buffer IX
  - 3 µL kB Ladder
- NOTE:
  - Test Tubes, 1-5 Yoyo miniprep (4/4)
  - 1a + 2a Elaine and Sunny miniprep (4/5)

LANE	
3	1 kB Ladder
4	Standard (Lineralized SS plasmid)
5	K331009-RFP-DT
6	2 “
7	3 “
8	4 “
9	5 “
10	6 LUC BSB1AT3
11	7 K331009-RFP-DT (1a)
12	8 “(2a)

Loading and Running Agarose Gel (Continued)

- 135 V for 35 min – Inconclusive results
- Restriction (Before the Gel)
  - 3 µL milliQ H2O
  - 1 µL Buffer
  - 5 µL DNA
  - 1 µL Enzyme

Samples	1-5 Yoyo miniprep 4/4
1	K331009-RPF-DT
2	"
3	"
4	"
5	"
6	"
7	"
8	"

Restriction of K331009-RFP-DT 1a and 2a

- Date: April 7, 2014
- Names: Yoyo

- 5:33pm START
  Incubated @ 37˚C for 1 hr

Components	Volume (µL)
MILLI-Q H2O	3
10 Cutsmart Buffer	1
pDNA (K331009-RFP-DT 1a/2a	5
Xba1	1
Total	10

Running 1% Agarose Gel to Check Previous Ligation

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

- Run a 20mL agarose gel with 4 wells used (1% gel)
- Materials for gel
  - 20mL 1x TAE
  - 0.2g of agarose powder
- DNA Samples (1a, 2a from Saturday April 15)
  - 2 µL Loading Dye
  - 10 µL DNA
- 1kB Ladder
  - 1 µL Loading Dye
  - 2 µL TAE Buffer 1x
  - 3 µL 1kB Ladder
- Standard (Lineralized SS plasmid)
  - 1 µL Loading Dye
  - 2 µL TAE Buffer 1x
  - 3 µL Standard

- **Standard (BBa-K331009) PMA 19
- Run Electrophoresis @ 135V for 45 mins
- If
  - Yes, set up overnight restriction of promoter J040500 with
  - No…pick more cells (D: Oh noouuu!)
- Stain for 20 min in EtBr
- De-stain for 5 min

Lane	
2	1kB Ladder
3	Standard(Lineralized SS plasmid)
4	1a (K331009-RFP-DT)
5	2a “

Picking Cells for 5 Colonies

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

- Note: Added 5 µL of AMP (100mg/mL)
- Procedure:
  - 1.5 LB Tubes retrieved
  - 2.5 µL of AMP added
  - 3 Leave to incupate @ 37˚C (RNA common room/ overnight)
- Colonies Picked → Labeled on Dish
- Number of colonies on LB media tube

	Stock
1.	Tube + K331009 + RFP + DT
2.	" "
3.	" "
4.	" "
5.	" "

Digesting Miniprep (05/04/14) at S,P

- Date: April 8th 2014
- Names: Dinula and Wesley

- Minipreps of:
  - 1a RFP – DT 05/04
  - 2a RFP – DT 05/04

- Tube Labelled

  K331009 +KFP + DT
  Xba1 Pst1
  Dinula De Silva
  05/04/14

Components	Volume (µL)
Milli-Q H2O	8
10X Cutsmart Buffer	2
pDNA	8
Xba1	1
Pst1	1
Total	20

Preparation of Agarose Gel

- Date: April 8/14
- Names: Sally and Sunny

- Added 1.00g of Agarose A to 100mL of 1x TAE
- Weighed 198.65g
- Microwaved for 80 seconds in 20 sec
- Increments then changed to 10 second
- Increments, until completely dissolved
- Added distilled water to maintain mass
- Poured the gel solution into 2 moulds

- *Ran at 135V for 30min 1x TAE
- Both bands moved lower from the well. Perhaps need less voltage or deeper wells. Maybe back to .5x TAE. Stopped get at 22 min.

Miniprep of BBA_K331009-RFP+DT

- Date: April 8/2014
- Names: Roya and Christina
- Procedure:
  1. 3 mL of culture to 1.5 mL tubes
  2. Centrifuged at 12 000 rpm for 2 mins
  3. Discarded supernatant into waste beaker
  4. Added 100 µL of solution 1 and resuspended
  5. Added 200 µL of solution 2 and inverted
  6. Added 350 µL of solution 3 and mixed gently
  7. Centrifuged at 12 000 rpm for 5 mins
  8. Transferred supernatant to EZ-10 column
  9. Centrifuged at 10 000 rpm for 2 mins
  10. Discarded flow through
  11. Added 750 µL of wash solution and centrifuged at 10 000 rpm for 2 mins (2X)
  12. Discard flow through
  13. Centrifuge at 10 000 rpm for 1 min
  14. Transferred column to clean 1.5 mL tube
  15. Added 50 µL elution buffer and centrifuge at 10 000 rpm for 2 mins
  16. Stored DNA n -20 ˚C

Digest of K331009+RFP-DT

- Date: April 9, 2014
- Names: TDang, Wes and Yoyo
- Digestion (same as one on April 8)
  - 8 µL d2H2O
  - 2 µL 10 X Cutsmart Buffer
  - 8 µL pDNA
  - 1 µL XbaI
  - 1 µL PstI
- Make digestion
- Incubate at 37 ˚C for an hour
- While incubating prepare a 40 mL 1% agarose gel
  - 0.4 g agarose gel
  - 40 mL TAE 0.5 X

Lane	DNA
1	Bba-K331009+RFP-DT
2	“
3	“
4	“
5	“

Running Agarose Gel Digested DNA Continued…

- Date: April 9, 2014
- Names: TDang, Yoyo and Wes

- Goal: When we run digested from other colonies and they prove successful, purify them or get more samples from the colony.
- Use 1xTAE for electrophoresis
- Sample Volume (Total 6 µL)
  - 5 µL sample pDNA
  - 1 µL loading dye
- **40mL agarose gel too thin that comb went through doing a 50mL gel
  - 50mL TAE 0.5x
  - 0.5g agarose powder
- Run gel 30 minutes at 135V
- Stained gel in EtBr for 20 minutes
- De-stained for 5 minutes
- **Poured 1xTAE from electrophoresis into 0.6x TAE bottle
- Results: Under the UV light lane 7,9,10 (Tubes 1,3,4) showed 2 bands; the band lanes showed that the ligations worked.

Lane	DNA
6	Ladder
7	1Bba-K331009
8	2 “
9	3 “
10	4 “
11	5 “

Restriction K331009+RFP+DT

- Date: April 10, 2014
- Names: Dinula, Sunny and TDang

- Objective: To determine the resistance on the backbone by placing the transformation on 3 different dishes (AMP-ampicillin, CAM-Chloraphenical, KAN, Kanamycin)
- Concentration of Ligated DNA
  - K331009+RFP+DT = 44.1ng/µL
  - K331009+RFP+DT = 45.2ng/µL
  - K331009+RFP+DT = 46.6ng/µL
- Tubes
  - 1: K331009+RFP+DT from April 8 (Tubes labeled April 10)
  - 3: "
  - 4: "
- Issue: For the J04500, the backbone is unknown as it was not labeled on the tube. As a result dilute the initial concentration of J04500 and digest. After, ligate the J04500 and K331009+RFP +DT and transform.

Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Xba1	0.5
Pst1	0.5
Total	20

Maxi prep for J04500/ Restriction of J04500 Continued…

- Date: April 10, 2014
- Names: Dinula, Sunny and TDang

$C_1V_1=C_2V_2$
25000ng/µL (V_1)= 50ng/µL (500µL)

For maxi prep prepare 100µL volume
50ng/25000ng= 1µL/500µL

For solution (total 100µL)
99.8µL mili q ($D2H_2O$)
0.2µL J04500

- After dilution, use the same procedure for digestion on April 10, 2014
- Incubate overnight 37˚C

Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Spec1	0.5
Pst1	0.5
Total	20

Gel Extraction

- Date: April 11, 2014
- Names: Dinula, Zak, Wes and Christina

- 40ml 1% Agarose in used 1xTAE made with 1x TAE
- Gel Procedure notes
- Sample 1 and 3 only yielded 16 µL of DNA solution from yesterdays restriction. We have no idea how this may have happened, sample 4 appears to have normal volume
- 100V for 50 mins
- *TOO LONG, BANDS RAN OFF GEL*
- Redoing Restriction

Lanes	
1	LADDER
2	Sample 1 K331009+RFP+DT 20µL
3	Sample 3 “
4	Sample 4 “

Restriction of K331009+RDP+DT and J04500 (Continued)

- Date: April 11, 2014
- Names: Wesley, Christina and Zak

- ALL WERE FINISHED
- At 7:40pm and placed in the RNA common room incubator

K331007-RFP-DT	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	15
Xba1	1
Pst1	1
Total	20

J04500	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	15
Spec1	1
Pst1	1
Total	20

Gel of K331009+RFP+DT

- Date: April 12, 2014
- Names: Sunny, Tiffany, Chris, Wes, Christina and Zak

- Made with 1x TAE and ran in used 1x TAE at 135V for 25 min (40mL)
- DNA Sample
  - 20 µL DNA
  - 4 µL loading dye
- Ladder
  - 5 µL 1KB Ladder
  - 1 µL loading dye
- Stained in ETBr for 25 minutes (total)
- Could only see one band at 2500bp instead if 800bp (BAD)

Lanes	
1	Ladder
2	Sample 1 K331009+RDP +DT
3	Sample 3 “
4	Sample 4 “

Picking (Continued)

- Date: April 12, 2014
- Names: Sunny, Tiffany, Chris, Wes, Christina and Zak

- Picking
  - Glycerol stock of K331008
  - Ligation Colonies 1,3,4
  - K331009+RFP+DT
- Antibiotic: Ampicillin
- Vector: pMAT
- Cell: DHSα

- Cells picked form plate and glycerol stock
- Put it in the shaking incubator at 37˚C overnight

Tried to gel extract K331009+RDP+DT but we couldn't see the 800bp on our gel. We made more ligation cells to try the gel extraction again.

Miniprep of ligation colonies 1,3,4, and K331009 and K331008 glycerol stocks

- Date: April 13, 2014
- Names: Zak

- Step by step mini prep- all steps done to each of the 5 µL cultures
- *Before starting, put elution buffer in 60˚C oven

1. 2 µL added to centrifuge tubes
2. Spin 12000rpm for 2 minutes
3. Drain liquid
4. 2 µL liquid added to centrifuge tubes
5. Add 100 µL solution 1 in RNASE and pipette up and down
6. Let sit for 1 minute at room temperature
7. 200 µL Solution 2 added. Flip the tube upside down a few times
8. Let sit for 1 minute
9. 350 µL Solution 3 added, tube flipped a few times
10. Centrifuge at 12000rpm for 5 minutes
11. Set p1000 to 700 µL and took up clear supernatant
12. Make sure to avoid taking up any white solid (genome)
13. Supernatant added to labeled mini prep columns, spin 10000rpm for 2 minutes
14. removed flow through
15. Added 750 µL wash solution, spun 10000rpm, 2 minutes. REPEATED
16. Spun again for 1 minutes to get rid of extra wash
17. Warmed Elution Buffer added to column (40 µL)
18. Columns left in 60˚C oven for 2 minutes
19. Spun at 10000 rpm for 2 minutes
20. Done! Tubes in -20˚C HS iGEM 2014 box.

Nanodrop of minipreps from April 13/14

- Date: April 14 2014
- Names: Zak

- We wanted to find the concentration of plasmid minipreps of our successful ligations and signal sequence plasmids.
- Taken to CCBN
- All 260/X ratios are good, so purity is good.

Miniprep	Concentration (ng/ µL)	260/280	260/230
Ligation 1	250	1.93	1.68
Ligation 3	393	1.93	1.94
Ligation 4	233	1.92	1.69
K331009	403	1.92	1.97
K331008	387	1.93	1.98

Digestion of J04500 and K331009

- Date: April 14 2014
- Names: Yoyo

- 1 tube of J04500 (Spec 1/ Pst 1)
- 3 tubes K331009+ RFP – DT
- Digested in anticipation of gel. Extraction/ quantification and ligation beyond this point.

Components	Volume (µL)
MILLI-Q H2O	12
10 Cutsmart Buffer	1
pDNA (~100µg/ml)	5
Enzyme I	1
Enzyme II	1
Total	20

Digestion of J04500 and K331009

- Incubate at 37 degrees Celsius for 3-4 hours.
  Start time : 4:13pm

J04500	
Components	Volume (µL)
Milli-Q H2O	12
10X Cutsmart Buffer	1
pDNA (Ligation Miniprep)	5
Spec1	1
Pst1	1
Total	20

K331007-RFP-DT (Colonies 1, 3, 4)	
Components	Volume (µL)
Milli-Q H2O	12
10X Cutsmart Buffer	1
pDNA (Ligation Miniprep)	3 X 5 (k331009-RFP-DT)
Xba1	1
Pst1	1
Total	20

Running Gel on Ligation Colonies from April 13, 2014

- Date: April 14, 2014
- Names: Christina, TDang, Chris, Yoyo, Wes and Kieran

- Objective: Today running a gel to see if the digestion done earlier today worked. Hopefully after running the gel we will se a band for 800bo and 2500bp for the backbone.
- Gel preparation of 1% Agarose Gel
  - 40mL 1xTAE Buffer
  - 0.4g Agarose
- 1KB Ladder
  - 5 µL 1 KB Ladder
  - 1 µL Loading dye
- Samples (Culture 1,3,4) and Promoter
  - 20 µL of DNA
  - 4 µL Loading dye

- Note: J04500 had a volume too large for a small well and so will be in lanes 6-7
- Run electrophoresis at 135V for 25 minutes
- Stain 5-10 minutes
- De-stain 20 minutes

Lanes	
1	1Kb Ladder
2	Culture 1 (K331009+RFP +DT)
3	Culture 3 “
4	Culture 4 “
6-7	J04500

Running Gel on Ligation Colonies from April 13, 2014 Continued…

- Date: April 14, 2014
- Names: Christina, TDang, Chris, Yoyo, Wes and Kieran

- Note: Gel Extraction Tips
  - Elute with H2O (Milli Q) → Not Elution Buffer
  - Warm water in 60˚C oven → out in when you start the gel
  - At final elution step, add warm water and let columns sit in 60˚C oven for 2 minutes
- Purifying and Extracting DNA from digested DNA that was run on April 14 gel.
- Record mass of the tube without the gel
- Using the long wave UV light cut the DNA band for 800bp (lowest band) and place in respected tubes
- Weigh tubes with cut DNA and record mass
- Running out of time and its late will run extraction tomorrow. The tubes with gel stored at 4˚C

Tube	Initial	Final	Gel Mass	Binding Buffer
1	1.13g	1.50g	0.37g (370mg)	1480µL
3	1.13g	1.50g	0.37g (370mg)	1480µL
4	1.13g	1.48g	0.35g (350mg)	1400µL

Gel Extraction

- Date: April 15, 2014
- Names: Sunny and Chris

- Procedure:
  1. Tube Binding Buffer II
  2. 1 1480 µL
  3. 3 1480 µL
  4. 4 1400 µL
  5. 2. Incubated at 60˚C for 15 minutes
  6. 3. Added mixture to the EZ 10 column in 3 separate doses (800 µL, 800 µL, what's left) and centrifuged at 10,000rpm for 2 minutes after each
  7. 4. Added 750 µL of wash solution, centrifuged at 10,000rpm for 1 minute. Repeated again.
  8. 5. Centrifuged without wash solution again.
  9. 6. Added 50 µL of Milli Q H_2O to the center of the column and incubated for 2 minutes at 60˚C
  10. 7. Centrifuge for 2 minutes at 10,000 rpm
- Stored DNA in -20˚C in iGEM Lab
  1: D1
  2: D2
  3: D3

Making Agar and LB media

- Date: April 15, 2014 (7-9pm)
- Names: Roya, Hope, Peter, Chris and Elaine

- As per recipes outlined on first page of Green Binder
- For First Recipe:
  - 3 g Trypton
  - 1.5 g Yeast
  - 3 g NaCl
  - 270 mL H_2O

Testing pH of LB agar jars and 5mL LB media tubes; Ligation of K331009-RFP-DT

- Date: April 16, 2014
- Names: Yoyo and TDang

- Bring pH of LB tubes and agar jars to 7 using NaOH in Wieden Lab-our pH meter is broken
- Ligation of K331009-RFP-DT and J04500
- *Note: 3:1 ratio of insert to vector

Components	Volume (µL)
Milli Q H2O	4
10x Buffer (T4 Ligase)	1
DNA1 (K331009+RFP +DT)	3
DNA2 (J04500)	1
Ligation Enzyme	1

Digestion of K31008 and RFP-DT (Continued…)

- Date: April 16, 2014
- Names: Yoyo and TDang

- If our ligation of K31009-RFP-DT does not work we are going to try K331008 instead as our DNA
- The tubes for K331008 digestion are labeled with 1 or 2 corresponding to the tube it was from (we used 2 different tubes of K331008)
- *Incubated overnight at 37˚C in E770
- Same recipe as digestion on March 24, 2014

Components	Volume (µL)
Milli Q H2O	4
10x Cut smart Buffer	1
pDNA (K331008)	4
Enzyme 1 (Spec1)	0.5
Enzyme 2 (Pst1)	0.5

Components	Volume (µL)
Milli Q H2O	4
10x Cut smart Buffer	1
pDNA (RFP-DT)	4
Enzyme 1 (Xba1)	0.5
Enzyme 2 (Pst1)	0.5

(Continued) Ligation of K331009-RFP0-DT and J04500

- Date: April 16, 2014
- Names: Yoyo and TDang

- Note: The tubes labelled in red are from a smaller tube with an open plasmid. Also the black tubes might have J04500 S,P in E3
- Note: K331009-RFP-DT was taken from colonies 1 and 3 from extraction on April 15 and are denoted on tubes for both colours.

Components	Volume (µL)
milliQ H2O	4.75
10x Ligation Buffer	2
DNA k331009-RFP-071	10
DNA (704500)	1.25
Ligation Enzyme (T4 DNA Ligase)	2

(Continued) Ligation of K331009-RFP0-DT and J04500

- Ligation set overnight in 16˚C thermocycler in Wieden Lab

- Concentration of Ligations
- Assumed concentration of J04500: 400µg/µL
- Assumed concentration of K331009+RFP-DT: 15µg/µL
- 3:1 Ratio of DNA wanted (C=n/v)

- 40µg/µL = n/1.25µL => n= 50ng of J04500
- 15ng/µL = 150ng/v => v= 10µL of K331009+RFP-DT
- 3:1 Ratio of K331009+RFP-DT to J04500
  10µL 1.25µL

- Special thanks to Dylan for saving our ligations tonight!

RFP-DT Digestion

- Date: April 17, 2014
- Names: Ronja and Dinula

Components	Volume (µL)
Milli Q H2O	7
10x Cut smart Buffer	2
pDNA (RFP-DT)	10
Xba1	0.5
(Pst1)	0.5

- Same protocol as pg. 24-25 of lab book
- Overnight digest @ 37˚C ET10

Transformation of J04500+K331009+RFP-DT (colony 1+3)

- Date: April 17, 2014
- Names: Ronja and Dinula

- Procedure
  1. Heat kill ligase
     1. -> 65˚C for 10mins
     2. centrifuge @10,000 rpm for 2 mins
  2. Grabbed two 20µL of pre dliquotted cells from -80˚C freezer thawed
  3. Pipetted 1.8µL of DNA into each tube into the competent cells and pipette once up and down to rinse the tip
  4. Mix the DNA into the cells by swirling the tip in the solution
  5. Incubate the cells on ice for 30mins
  6. 6) Heat shock the cells in water bath at 42˚C for exactly 45 seconds
  7. Add 250µL of sterile media to the cells and incubate at 37˚C for 1 hour with shaking. Tape microcentrifuge tube in shaking incubator.
  8. Label the LB plates and plate 83 µL of cells on each plate CKAN, CAM, AMPS for both tubes
  9. Leave plate for 10-15 min to soak the cell suspension into the agar
  10. Flip plate over with agar on top and incubate at 37˚C oven RNA common room overnight
  11. Keep the remaining solution in the 4˚C fridge overnight until transformation has been confirmed

1% Agarose Gel

- Date: April 18, 2014
- Names: Dinula, Ronja, Yoyo

- Objective: Today running a gel to see if the digestion odne yesterday worked. If it worked, we should se a band for 600-800bp
- Gel preparation of 1% Agarose gel
  - 40 mL 1x TAE Buffer
  - 0.4 g Agarose
- 1kb ladder
  - 5 µL 1kb ladder
  - 1 µL loading dye
- Samples
  - 20 µL of DNA
  - 4 µL of Loading dye
- 6x Loading dye ran out after lane 4, lane 5 has another dye
- Note: lanes 2 and 3 only contained 20 µL and lanes 4 and 5 contained slightly less than 20 µL
- Run electrophoresis at 135V for 25 min
- Stained in ET Br for 12 mins
- Destained for 20 mins

Lane	
1	1kb Ladder
2	Culture 1 (K33008)
3	RFP-DT
4	RFP-DT(1)
5	RFP-DT(2)

- Note: We went to go see our bands under UV light in Wieden Lab… the UV light box wasn't there. After ~40mins the UV light box was in E770.
- Note: No picture as the bands didn't show on my camera-phone (sorry!)
- Lane 2's band was ~2500-2000 bp
- Lane 3-5's lower band was ~1000-750bp

Picking cells- Transformation

- Date: April 18
- Names: Yoyo, Ronja and Dinula

- *Worked in open flame
- Cells grew in t he KAN and AMP antibiotic plates.
- To make sure we have our DNA, we picked both plates
  - 2x KAN plates w/ cells (colony 1 and 3)
  - 2x AMP plates w/ cells (colony 1 and 3)

- We picked three times per plate
- Picking spots were also labeled on the plate
- LB Media Tubes are labeled:

  E.coli NEBα J04500+ BBa-K331009+RFP+DT (April 18 Colony 1 or 3)

  E.coli NEBα J04500+ BBA-K331009+RFP-DT (April 18 Colony 1 or 3)
- With pick spot labeled as well
- Incubated at 37˚C in RNA Common Room overnight

Extracting and Purifying DNA from April 18 Gel

- Date: April 18, 2014
- Names: Yoyo

	Tube	Initial (g)	Final (g)	Gel Mass
Lane 2	2	1.04	1.25	0.21g => 210mg
Lane 3	3	1.03	1.27	0.24g => 240mg
Lane 4	4	1.05	1.31	0.26g => 260mg
Lane 5	5	1.0	1.43	0.39g => 390mg

Tube 2	210mg x4	840µL of Binding Buffer 11
Tube 3	240mg x4	"
Tube 4	260mg x4	"
Tube 5	390mg x4	"

(Continued) Extracting and Purifying DNA from April 18 Gel

1) Cut gel w/ appropriate DNA fragment, transferred into a 1.5mL microfuge tube
2) Added the tube's respective amount of Binding Buffer II. Incubated at 60˚C for 10 mins, shaking occasionally
3) Added step 2 solution into EZ-10 Columns. Added in 800 µL increments, to avoid overflow. Let stand for 2 mins, centrifuge at 10,000 rpm for 2 mins, repeated until all solutions were centrifuged. Discarded the flow-through.
4) Added 500 µL of Wash solution, centrifuged at 10,000rpm for 1 min. Discarded solution in tube
5) Added 500 µL of Wash solution, centrifuged at 10,000rpm for 2 min. Discarded solution in tube
6) Placed blue column in clean 1.5mL microfuge tube. Added 35 µL of d2H2O (at 60˚C) onto center of the column. Incubate at 60˚C for 2min
7) Centrifuged at 10,000rpm for 2min. Discard EZ-10 column's. Purified DNA stored in -20˚C fridge

Tube 2	E4	K331008 Cut S,P Purified
Tube 3	E5	RFP-DT Cut X,P " _____"
Tube 4	E6	RFP-DT(1) Cut X,P "_____"
Tube 5	E7	RFP-DT (2) Cut X,P "_____"

Miniprep of J04500+K331009/RFP-DT from April 18th

- Date: April 19, 2014
- Names: TDang, Sunny, Wes, K-Dawg, Dinula, Ronja

- *NOTE: Culture 3x1 was spilled and only 1mL was mini-prepped
- Protocol
  1) 2mL of cultured cells were added to microfuge tubes
  2) Cells were spun at 1200rpm for 2mins and liquid was discarded
  3) Repeat steps 2 and 3 adding equal volumes to their respective tubes, centrifuging and draining
  4) Add 100 µL solution I (with RNASE) to microfuge tubes 8 pipette up 8 down
  5) Sit ofr 1 min at room temp
  6) 200 µL of solution II added. Flip upside-down a few times
  7) Sit for 1 min at room temp
- Continue from p. 29 protocol
- Started protocol before finding old one, oops
- NOT ALL CULTURES WERE 5mL, THIS MAY RESULT IN VARYING GEL CONCENTRATIONS

DIGEST OF MINIPREP (Linerization of ligated J04500+K331009/RFP-DT)

- Date: April 19, 2014
- Names: TDang, Sunny, Wes, K-Dawg, Dinula, Ronja
- 13 Samples digested at X
  - 1 J04500 plasmid (1/100 dilution)
  - 6 KAN samples
  - 6 AMP samples

Components	Volume (µL)
Milli Q H2O	3
10x Cut smart Buffer	1
pDNA	5
Xba1	1

- Incubate for 1 hour 37˚C

Gel of Miniprep of J04500+K331009/ RFP-DT from April 18th

Gel Confirmation

- 13 lanes
- 1% gel made w/ 1x TAE ran in 1x used TAE 10 µL pDNA/2 µL loading dye in each lane
- 135V – 45 minutes
- Stained in ET Br for 20mins, destained for 5mins
- Samples may have run off of the gel

Lane			
1	Ladder	9	1x1 KAN
2	Blank	10	1x2 KAN
3	1x1 AMP	11	1x3 KAN
4	1x2 AMP	12	3x1 KAN
5	1x3 AMP	13	3x2 KAN
6	3x1 AMP	14	3x3 KAN
7	3x2 AMP	15	Blank
8	3x3 AMP	16	Linear J04500
		17	Blank

Pipetting tips

- Date: April 21, 2014
- Names: Tiffany D., Christina

- 6 of 0.1 -10 µL Micro Tips => (Bio Basic Inc.)
- 1 of 0.5-10 µL Fisherbrand => Micropipette Tips

(Gel Extraction cont.) Digesting RFP-DT and K331008

- Date: April 21, 2014
- Names: TDang and Christina

- The concentrations for the RFP-DT and K331008 were Very low and so we are going to digest them and hope to improve concentration
  - K331008 S+P = 0.9 ng/ µL
  - RFP-DT X+P = 0.8 ng/ µL
- However we are only using 1 tube of the K331008
- Note: There was no RFP-DT so we will need to still digest
- Incubate the K331008 overnight @ 37˚ in E770

K331008	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Spec1	1
Pst1	1
Total	20

RFP-DT	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Xba1	1
Pst1	1
Total	20

Digestion of RFP-DT and K331008

- Date: April 22 2014
- Names: Chris, Dinula, Ronja

- Incubate K331008 and RFP-DT for 2 hours in room E770
- Restriction of K331008 and RFP-DT
- Heat killed and placed in -20˚C fridge by Z.S.
- I1 and I2 in fridge

K331008	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Spec1	1
Pst1	1
Total	20

RFP-DT	
Components	Volume (µL)
Milli-Q H2O	7
10X Cutsmart Buffer	2
pDNA (Ligation Miniprep)	10
Xba1	1
Pst1	1
Total	20

Picking Stocks

- Date: April 22, 2014
- Names: Elaine, TDang, Tiffany and Sunny

- 6 X 5µL of LB Media (PSB1C3 with CAM in a 1:1000 ratio)
- Picked frozen stocks and placed it in the shaker at 37˚C overnight in the RNA Common Room

Genewiz Sequencing for < 6 KB plasmids

- Date: April 22, 2014
- Names: Ronja, Christina and Chris

- 500 ng/10 µL pDNA
- 25 pmd m 5 µL primers
- 15 µL solution total

- DNA sent out for sequencing on the morning of April 23

- $C_1V_1 = C_2V_2$

Ligation	Concentration (ng/µL)	Volume DNA (µL)	Volume H20 (µL)
K1.1	139.6	3.58	6.42
K1.2	193.8	2.58	7.42
K1.3	252.0	1.98	8.02
K3.1	90.0	5.56	4.44
K3.2	104.3	4.79	5.21
K3.3	131.4	3.81	6.19

1% Agarose Gel

- Date: April 23, 2014
- Names: Dinula and Yoyo

1% Agarose Gel

- Note: Lane 2 and Lane 3 were from April 22
  - 21 µL DNA
  - 4 µL 6X Dye

- For the Ladder
  - 1 µL Dye
  - 2 µL of 1X TAE
  - 3 µL of 1 KB Ladder

- Ran 135 V for 30 Mins
- Stained in EtBr for 20 mins
- De-stain for 10 mins

- Note: Gel was unsuccessful. Need to remember to keep gel in plastic molding container when running gel.

Lane	
1	Ladder
2	K331009 Cut at S and P
3	RFP-DT Cut at X and P

Miniprep RFP-DT + PSBiC3 from April 22 Overnight Stocks

- Date: April 23, 2014
- Names: Dinula, Ronja, Yoyo and Wesley

- Step by Step miniprep –all steps done to each of the 5 mL cultures (** some where 3-4 mL)
- Before starting, put elution buffer in 60˚C oven
- 1.5 mL added to centrifuge tubes
- Spin 12 000 rpm for 2 minutes
- Drain liquid
- 1.5 mL liquid added to centrifuge tubes
- Add 100 µL Solution 1 with RNASE and pipette up and down
- Let sit for 1 minute@ room temperature
- 200 µL of Solution 2 added. Flip the tube upside down a few times.
- Let sit for 1 minute.
- 350 µL Solution 3 added, tube flipped a few times
- Centrifuged @ 12 000 rpm for 5 mins
- Set p100 to 700 µL and take up all clear supernatant
  - Make sure to avoid taking up any white solid (genome)
- Supernatant added to labeled miniprep columns, spin 10 000 rpm for 2 mins
- Removed flow through
- Added 750 µL of wash solution spun 10 000 rpm for 2 mins and repeat
- Spun again for 1 minute to get rid of extra wash
- Warmed Elution Buffer added to column (40 µL)
- Columns left in 60˚C oven for 2 mins
- Spun 10 000 for 2 minutes
- Done! Tubes in -20˚C HS iGem 2014 box

Restriction of RFP-DT + K331008

- Date: April 23, 2014
- Names: Dinula, Ronja, Wesley and Yoyo

- Objective: Obtain gene of interest for later ligations
- Overnight digestion in 37 ˚C Incubator in E770. We used the black #1 RFP-DT tube from previous miniprep. In incubator @ 6:40 pm April 23
- Note:
  - RFP-DT – X and P
  - K331008 – S and P

Components	Volume (µL)
Milli Q H2O	9
10x Cut smart Buffer	2
pDNA	3
Enzyme 1	0.5
Pst1	0.5
Total	20

1% Gel of RFP-Dt + K331008

- Date: April 24, 2014
- Names: Roya, Wesley and Zak

- Made 30 mL of 1% Agarose Gel
- Made with 1 X TAE
- Ran in 1X TAE
- Ran at 100 V for 30 mins

- Added 5 µL of Chloramphenicol to 6 5 mL media tubes
- Incubated 6 X 5 mL culture tubes with cells containing RFP-DT plasmid
- Put in @ 9 pm

Lane	Ladder
1	Ladder
2	K331008 (Cut @ S and P on April 23)
3	Black/RFP-Dt (Cut @ X and P on April 23)
4	None

Miniprep of RFP-DT

- Date: April 26, 2014
- Names: Sunny, Ronja, Dinula, TDang and Zak!

- Follow procedure on pg 29 of the lab book (April 13/14) previous concentrations of last miniprep were very low
- Running gel to confirm minipreps
- ** 20 mL gel (1 X TAE)
- Note: When loading the lanes, the DNA did not go down –it floats up??? Could have been the fact that the gel did not solidify completely when comb was taken out. Did not run gel.

Lane	
1	1 KB Ladder
2	Red Tube 1 RFP-DT
3	" Tube 2 "
4	" Tube 3"
5	Black Tube 1 RFP DT
6	" Tube 2 "
7	" Tube 3"

Nanodrops of April 26 Minipreps

- Date: April 28, 2014
- Names: Zak

- RFP-DT 1 (red) -320.5ng/µl
- RFP-DT 2 (red) -198.6ng/ µl
- RFP-DT 3 (red) -301.1ng/ µl
- RFP-DT 1 (black) -338ng/ µl
- RFP-DT 2 (black)-278.2ng/ µl
- RFP-DT 3 (black) -323ng/ µl

Restriction of RFP-DT and K331008

- Date: April 28
- Names: Chris

- To restrict RFP-DT in preparation for ligation
- Transferred to incubate at 37˚C in E770 at 2:45
- Tried to run 1x Gel 135V of RTP-DT(1) but bands moved together.
- * Successful Extraction

Dilution of Stocks for Sequencing

- Date: April 29
- Names: Christina, Hope, Yoyo, Chris and Elaine

A	K331009 Glycerol MP (2)	270 ng/µL
B	K331009 MP (red writing)	60 ng/µL
C	K331009+RFP Lig (1)	236 ng/µL
D	K331009+RFP Lig (3)	334 ng/µL
E	K331009+RFP-dt Lig (4)	171 ng/µL
F	J04500 Max	25000 ng/µL
G	RFP-dt (1)	338 ng/µL

Sample	DNA (µL)	H2O (µL)
A1, A2	1.85	8.15
B1, B2	8.33	1.66
C1, C2	2.12	7.88
D1, D2	1.49	8.51
E1, E2	2.92	7.08
F1, F2	2.00	8.00
G1, G2	1.48	8.60

All samples 10 µL of 50ng/µL
Samples A2 → G2: DNA(µL) are all @2 µL
d2H2O (µL) are all @8 µL
Sample B2 → 8. 33 .. µL DNA + 1.8 … µL H2O
Sample C2 → 2.119 µL DNA + 7.881 µL H2O
Sample D2 → 2.92 µL DNA + 7.08 µL H2O

Dilution of Stock Cont

- Date: April 29, 2014
- Names: Dinula and Sunny

- JO4500 → Diluted to 250 ng/µL (100 mL) (maxiprep)
- Samples were sent for sequencing

Sample	DNA (µL)	H2O (µL)
A1, A2	1.85	8.15
B1, B2	8.33	1.66
C1, C2	2.12	7.88
D1, D2	1.49	8.51
E1, E2	2.92	7.08
F1, F2	2.00	8.00
G1, G2	1.48	8.60

Picking Glycerol Stocks

- Date: April 29, 2014
- Names: Dinula and Sunny

	Stock
1.	K331008 AMP April 29, 14 DHS α D.D. S.S.
2.	K331008 AMP April 29, 14 DHS α D.D. S.S.
3.	K331008 AMP April 29, 14 DHS α D.D. S.S.
4.	K331007 AMP April 29, 14 DHS α D.D. S.S.
5.	K331007 AMP April 29, 14 DHS α D.D. S.S.
6.	K331007 AMP April 29, 14 DHS α D.D. S.S.

Miniprep (pg 29 Protocol) of K331007/008

- Date: April 30, 2014
- Names: Wesley and Dinula

- K3301007/K331008 cultures from April 29 were miniprepped and left in the freezer in a green tube holder
- Please transfer to second HS iGem box when it has a sheet made
- pDNA to be tested fro concentration in the near future