PANAMÁ – INDICASAT 2012 NOTEBOOK

- **June 11, 2012**
    - **Members:** Paul & Ana
    - **Activities:** Lab work
- **June 14, 2012**
    - **Members:** Paul & Ana
- **June 15, 2012**
    - **Members:** Natasha
    - **Activities:** A Mini Prep was done for the RBS, GFP, Catechol and Terminator using the "Dirty Mini Prep Protocol." (2 Mini Preps per part).
- **June 18, 2012**
    - **Members:** Claudio & Dafne
    - **Activities:** A 40% Glycerol stock was prepared (50mL)
        - $C_1V_1 = C_2V_2 \rightarrow$ 100% V_1 = (40%)(50mL)
          V_1 = 20mL of glycerol in 30mL H_2O(d).

      We transferred the 8 plates to a liquid medium (LB)
      The results for the plates with Amp C^- and Clor C^- were OK.

Plates	Antibiotic
Catechol 1	Chloramphenicol
Cat 2	Chloramphenicol
RBS 1	Ampicillin
RBS 2	Ampicillin
GFP 1	Ampicillin
GFP2	Ampicillin
Ter 1	Ampicillin
Ter 2	Ampicillin

- We added 1mL of broth
  - 1mL of glycerol
  - 2mL in one cryovial

  Note: the final result is glycerol 20% after adding the broth.

We ran an electrophoresis of the Mini Prep done by Natasha.

We added 4µL of the sample

- 2µL of Loading Buffer
- 2µL of the Weight Ladder

Lane	Part
1	Weight Ladder
2	GFP 1
3	GFP2
4	Cat 1
5	Cat 2
6	Ter 1
7	Ter 2
8	RBS1
9	RBS 2

June 20, 2012

- **Members:** Natasha & Elkjaer
- **Activities:** We did a transformation of the constitutive promoter
    - Prepared LB medium: 1.5g of LB in 75mL
    - We took 10µL of molecular H_2O and transferred it to the N15 well (constitutive promoter).
    - We labeled 3 tubes:
        - 1. T7 Constitutive Promoter
        - 2. DNA
        - 3. Negative Control (C-)
    - After 10 minutes, we took 10µL of the constitutive promoter (T7) and transferred it to tube 1 (T7)
    - We took the competent cells and transferred 50µL to tube 2 (DNA) and 50µL to tube 3 (C-). Everything was kept and done in ice.

- We transferred 2µL of tube 1 (T7) to tube 2 (DNA). C- is not included in this step. We left it in the ice for 30 minutes (T7 + DNA, tube 2 and C-). Then, we kept both in a temperature of 42°C for 1 minute and 5 minutes in ice. After that process, we added 200µL of SOC medium (to both tubes). Shake for 1 hour, 37°C.
- Autoclave LB medium. Then, we took out 5µL of the medium to one of the plates. We added Ampicillin to the LB medium and divided the medium into 12 plates.
    - [ ]Amp= 50µg/ml→LB
      (70µl Amp)

June 21, 2012

- **Members:** Dafne, Claudio, Orlando & Emily
    - Digestion of the RBS, GFP, Cat 1, Ter 1.
    - Transferred T7 promoter cultures to a liquid medium and cultures with no plasmid to make competent cells.
    - An electrophoresis was done by Dafne with:
    - M 100pb — 2µL Loading buffer
    - Cat (Catechol) x (1) — 2µL Weight Ladder
    - Ter — 10µL Samples
    - GFP
    - RBS

*Claudio & Emily met Denise LaBuda from Learning Center via Skype.com.

June 22, 2012

- **Members:** Dafne, Elkjaer & Natasha

- **Activities:**
    - We transferred bacteria with T7 CP plasmid to glycerol,
    - The strain without plasmid was also transferred to glycerol in order to preserve them and use them later on as competent cells (made by us). Incubate in -80°C
    - Mini Prep ("Dirty Mini Prep Protocol") T7
    - Step 4: centrifuged 13 000rpm for 5 minutes
    - Step 25: Re-suspend in 30µL of TE
    - We also prepared TAE 1X (1L) from TAE 10X.
    - Electrophoresis:
        - M — 2µL of Loading Buffer
        - T7 — 2µL of M from 100 to 100
        - T7 — 4µL of Sample
        - T7*

The T7 is a hybrid of 2 different tubes of T7.

Results: OK However, T7 wasn't very visible.

June 22, 2012

- **Members:** Elkjaer & Natasha
- **Acitvities:**
    - Digestion of:

Tube	Part	Quantity
	Promotor T7 A	
4	EcoR1	**1µL**
4	Spe1	**1µL**
3	BSA	**1µL**
5	DNA	**10µL**
2	2 Buffer	**5µL**
1	H_2O	**33µL**
Total Volume		**50µL**

RBS	
Part	**Quantity**
Xba1	**1 µL**
Pst1	**1 µL**
BSA	**1 µL**
DNA	**10µL**
2 Buffer	**5 µL**
H_2O	**32µL**
Total Volume	**50µL**

GFP	
Part	**Quantity**
EcoR1	**1µL**
Spe1	**1µL**
BSA	**1µL**
DNA	**10µL**
2 Buffer	**5µL**
H_2O	**32µL**
Total Volume	**50µL**

Terminator	
Part	**Quantity**
Xba1	**1µL**
Pst1	**1µL**
BSA	**1µL**
DNA	**10µL**
2 Buffer	**5µL**
H_2O	**32µL**
Total Volume	**50µL**

-

- We incubated for 2 hours, 37°C.
- Deactivate enzymes at 80°C, for 20 minutes.
- We also did digestion of pSB1C3 and pSB1K3 (plasmids) where we will do a ligation of all the parts.
- The plasmids were digested with EcoR1 and Pst1 with the same quantity of the previous reactions.
- Ligation:

P. T7 with RBS to plasmid psB1C3	
Substance	**Quantity**
H_2O	**12.5µL**
T4 Buffer	**2.5µL**
C. Vector	**1µL**
P.T7	**4µL**
RBS	**4µL**
Ligase	**1µL**
Final Volume	**25µL**

GFP with Ter to plasmid psB1C3	
Substance	**Quantity**
H_2O	**12.5µL**
T4 Buffer	**2.5µL**
C. Vector	**1µL**
GFO	**4µL**
Ter	**4µL**
Ligase	**1µL**
Final Volume	**25µL**

Catecol and Terminator to plasmid psB1K3	
Substance	**Quantity**
H_2O	**12.5µL**
T4 Buffer	**2.5µL**
K. Vector	**1µL**
Catecol	**4µL**
Terminator	**4µL**
Ligase	**1µL**
Final Volume	**25µL**

- We incubated the reactions at room temperature for 30 minutes.
- Deactivated the enzyme at 65°C for 15 minutes.
- We stored the reactions at -20°C

June 25, 2012

- **Members:** Ben & Dafne
- **Activities:**
  - Kanamycin stock: 50µg/mL or 25µg/ml = 35g/ml in water
  - Transformation:
    - a.) T7 + RBS
    - b.) GFP + Ter
    - c.) Cat + Ter
  - We prepared 200mL of medium with agar for the plates.
  - [ ] = 25g (agar)/L
  - We also prepared Kanamycin stock: 5ml → [ ] = 35mg/mL
  - We filtered and got a total of 4mL stock.
  - Ben prepared plates with antibiotics:
    - a.) 50µL Kanamycin in 50mL of medium (6 plates).

- b.) 1500µL Chloramphenicol in 150mL of medium. (6 plates)

June 26, 2012

- **Members:** Orlando
- **Activities:**
  - Just transformation of Catechol with terminator resistant to kanamycin. The rest of the ligations were all resistant to Chloramphenicol and they didn't grow.
  - The cultures that did grow were stored at 4°C in order to transfer them to a liquid medium later on.

June 28, 2012

- **Members:** Dafne
- **Activities:**
  - We transferred the cultures from both plates that grew from Catechol + terminator (K+) into a liquid medium.
  - We used 14 tubes: 7 tubes from plate 1 and 7 tubes from plate 2.
  - Each tube had 5ml of liquid medium LB and 5µL Kanamycin.
  - We incubated the tubes and stored the plates again at 4°C.

Plate 1	Plate 2
3	13
14	6
2	11
9	7
10	8
1	4
12	5

June 29, 2012

- **Members:** Eljaer, Natasha & Dafne
- **Activities:**
    - Mini Prep of the 14 tubes with Cat + Ter (K+) and 1 tube for C- with protocol "Dirty Mini Prep."
    - Digestion of plasmids with Pst1 in order to linearize the plasmid.

Substance	Quantity
H_2O	**33µL**
Buffer 3	**5 µL**
BSA	**1 µL**
DNA (cat +ter)	**10 µL**
Pst1	**1 µL**
Final Volume	**50 µL**

    - PCR: 37°C →90 minutes
    - 80°C →15 minutes (until 3:30p.m.)

June 29, 2012 (p.m.)

- **Members:** Elkjaer & Tamara
- **Activities:**
    - Electrophoresis of the digestion and ligation.
    - Box 1 (concentration of 2%)

a.) Weight Ladder (K180)
b.) RBS
c.) GFP
d.) Cat
e.) Ter
f.) Plasmid C
g.) Plasmid K
h.) T7
i.) GFP + Ter
j.) Cat + Ter

a.) Weight Ladder

b.) T7 + RBS

- Box 2 (concentration of 1%) → must prepare more on Saturday.
- Electrophoresis of the mini prep cat + ter, 14 samples:

a.) Weight Ladder

b.) Cat + Ter

a.) Weight Ladder

b.) Cat + Ter is #14

c.) C-

d.) C-

- Modify the PCR reaction with Platinum blue super mix. The reactant was at 1.1X, it should be used at the proportion of 45µL from the mixture plus 5µL of the mixture with the primers and template.
- The enzyme TaqPol that was in the kit was linked with an antibody. A previous denaturalization must be applied.

*Marla's recommendation for a PCR

June 30, 2012

- **Members:** Dafne & Emily
- **Activities:**
  - Mini Prep electrophoresis
  - Linearization of Cat + Ter

Substance	Quantity	Tube
L Buffer	2 µL	**1**
Weight Ladder	3 µL	
#1-13	10µL	
Weight Ladder	3µL	**2**
#14	10µL	
C^-	**1 µL**	

- In #8, 4, band appeared.
- We prepared stock of 0.1M $CaCl_2$ (3.7g in 250mL H_2Og.m.)
- Competent cells: "How to make competent cells" protocol.
- We prepared 4 falcon tubes with 50mL of LB and 5mL of cells (1:100)
- 20 tubes of 200µL of competent cells

July 6, 2012

- **Members:** Katherine & Arleny
- **Activities:**
  - Kit: Position 1a from Plate 1, RFP.
  - Transformation (A) BBa_1
  - 3 plates: #1 and #2 were positive control, #3 was C-
  - We followed the protocol

July 9, 2012

- **Members:** Dafne
- **Activities:**
  - The competent cells transformed with red fluorescent protein.

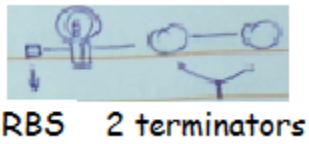
- We transferred to a liquid medium the white and pink cultures.
- (2 tubes)
  - Plate 1: Pink (tube 1)
  - Plate 2: White (tube 2)
  - Parts that we have used:
    - a.) Plate 1: iGEM kit 2012
      1a-RFP
      J5, K14, N15, L23
    - b.)Plate 4: iGEM kit 2012, C15

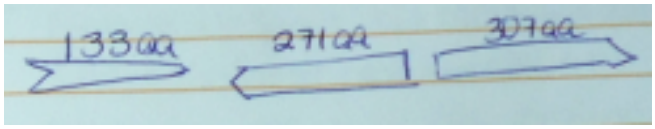
Code	Parts	bp + plasmid
1A	RFP	1069bp + BB
✓ 5J	RBS	12bp + pSB1A2
14K	GFP	720bp + pSB1A2 (1200bp)
✓ 15N	T7	23bp + pSB1A2 (1069bp)
✓ 23L	Ter	129bp + pSB1AK3 (1118bp)
***15C**	**Cat**	**1061bp + pSB1C3 (2070bp)**

Catechol

Well 15C

BBa_K316003





pSB1K3	Cat	Ter
2204bp	1061bp	129bp

bp	Plasmid
2079bp	**pSB1A2**
3189bp	**pSB1AK3**
2070bp	**pSB1C3**
2204bp	**pSB1K3**

- Restriction enzymes that cut T7

  a.) FaiI

  b.) FspEI

  c.) HinFI

  d.) MiyI

  e.) MspjI

  f.) PleI →4 sites

  g.) SfcI

- The Biobrick from well 1A→RFP has:

  a.) Promoter

  b.) RBS

  c.) RFP → 681bp BBa_E1010

  d.) Ter → 129bp BBa_B0015

  We could use RFP + Ter directly

- RFP alone→ Part BBa_E1010
- pSB2K3→Well 18F→ Plate 1 (2012)
- Both are Kanamycin resistant.
- We transformed competent cells with the Biobrick from well 18F (RFP).

  Plate 1- iGEM kit 2012

- We transferred from cryovial to liquid medium:

  a.) RBS

  b.) GFP

  c.) Ter

d.) T7

- We added 5µL to every tube with 200µL LB + Ampicillin (5µL).
- We transformed "homemade" competent cells with the protocol "Transformation" from 2011.igem.org with RFP.

  a.) We added 4µL of the plasmid (well 18F) in one eppendorf (with 200µL of cells)

  The other 6µL of plasmid were re-suspended and stored in one mini tube.

  b.) C^-: competent cells (200µL) with RFP (without plasmid).

  c.) We added 200µL of SOC medium to each tube. 2 eppendorf: RFP and the C^-.
- We incubated.
- We transferred directly to 2 falcon tubes with kanamycin K^+: RFP and C^-.
- We incubated overnight.

July 13, 2012

- **Members:** Orlando
- **Activities:**
  - Mini Prep of the promoter, RBS, GFP and RFP controls.

July 14, 2012

- **Members:** Katherine & Arleny
- **Activities:**

Parts	Enzyme
Promoter	**EcoR1 and Spe1**
RBS	**Xba1 and Pst1**
Reporter	**RFP and GFP**
Terminator	**Xba1 and Pst1**

- We added:

Substance	Quantity
NEB Buffer 2	**5µL**
BSA	**0.5µL**
Enzyme 1	**1µL**
Enzyme 2	**1µL**
DNA Mini Prep	**10µL**
H_2O	**32.5µL**
Final Volume	**50µL**

*Note: In this case we added 2 buffers because each enzyme has 1 (H_1B_1, etc.). In other words, 10µL of buffer→ 5µL each. We added less water (27.5µL) because we there were 2 buffers.

- We prepared 5 mini eppendorf with terminator (T), promoter (P), RBS (R), reporter (RFP and GFP).
- We put them in the thermocycler for 2 hours at 37°C and then we deactivated the enzymes at 80°C for 20 minutes.
- Electrophoresis: 1µL for each 50µL of agarose.
- To make the gel, we added:

Substance	Quantity
Agarose	**0.7g**
TAE 1X	**70mL**
Ethidium Bromide (after heating the mixture)	**2µL**

- To make the solution for the electrophoresis:

Substance	Quantity
Loading Buffer	**2µL**
Weight Ladder	**3µL**
Sample	**10µL**

- Order of the electrophoresis at 108V:

  4 ~ Weight Ladder

  5 ~ Promoter

  6 ~ RBS

  7 ~ GFP

  8 ~ RFP

  9 ~ Terminator

July 16, 2012

- **Members:** Elkjaer & Claudio
- **Activities:**
  - We digested the plasmid with resistance to Chloramphenicol with EcoR1 and Pst1.

Substance	Quantity
Buffer	**5µL**
BSA	**1µL**
EcoR1	**1µL**
Pst1	**1µL**
Vector	**1µL**
H_2O	**42µL**
Final Volume	**50µL**

  - We made a LB medium with agarose for 12 plates x 5mL. (Plus one extra)

65mL → 0.975g agar + 1.65g LB

- We did a ligation of:

Substance
RFP + T
GFP + T
RBS + P

July 17, 2012

- **Members:** Natasha
- **Activities:**
  - Deactivation of the ligation at 65°C for 15 minutes.

July 18, 2012

- **Members:** Claudio
  - The camera was not available.

July 20, 2012

- **Members:** Claudio
- **Activities:**
  - Transformation

July 20, 2012

- **Members:** Katherine and Arleny
- **Activities:**
  - We left on ice for 5 minutes, 6 tubes with competent cells.
  - Then we added 4µL of RBS + P, T+ GFP, T + RFP.

a.) 2 tubes with competent cells + RBS + P

b.) 2 tubes with competent cells + T + RFP

c.) 1 tube with competent cells + T + GFP

d.) 1 tube with negative control only with cells

- We incubated on ice for 30 minutes
- We did a heat shock at 42°C for 60 seconds. We left it on ice and incubated again for 5 minutes.
- We added 200µL SOC to the tubes (5). Incubated $1^1/_2$ hour at 37°C in a shaker.
- We spread 100µL of the cells that were transformed (5 tubes and left one as negative control) in one agar plate with resistance to Chloramphenicol
- Incubation at 37°C overnight.

August 4, 2012

- **Members:** Elkjaer & Dafne
  - We prepared medium:

Substance	Quantity	Distilled water
LB	25g/L	200mL
LB + Agar	25g/L + 15g/L	200mL
SOC		**100mL**

  - We prepared 7 plates of LB + Amp
  - We also prepared Amp stock → 50mg/mL
  - Glucose stock 2.0M for the SOC medium

Glucose 180.16g/m

$$2M \times \frac{180.16g}{1m} \times \frac{1.0L}{1000mL} \times 4mL = 1.44g$$

- We transferred the bacteria from a glycerol stock (2011) to a liquid medium (LB + Amp).

P + RBS

R + T

- Incubation

August 6, 2012

- **Members:** Katherine
- **Activities:**
  - Mini Prep using the same protocol except for step 3. We used 3 minutes instead of 1 min.
  - We did an extraction of 1.5mL in the room of extraction (didn't use the camera). We observed if there was any contamination.
  - The 3 tubes (negative control, T + R, P + RBS) were left in the pre-PCR refrigerator. The bacteria in liquid medium were left in the virology refrigerator.

  * Make glycerol stock for RFP!

August 15, 2012

- **Members:** Dafne
- **Activities:**
  - Possible parts from the ligations done by iGEM 2011:

Part	Location	bp
RBS	2M	34bp
Reporter	14K, E004	742bp
Ter	13D	
Promoter	15N, 1719005	45bp
pSB1A2	B0034	

*See June 6,7,8, 2011

- Pending:

  a.) Transformation with 2µL of RFP. Later on, make glycerol stock.

  b.) Electrophoresis of the Mini Prep that Katherine made (August 6)

  c.) Find out for sure the weight of P + RBS and R + T, used in 2011.

- The RFP (from well 18F) is at -20°C, virology room. It was a pink eppendorf labeled in red. It must have 6µL.

August 21, 2012

- **Members:** Natasha & Andres Nieves

$$C_iV_i = C_fV_f$$
$$= [35mg/ml] \times 5ml$$
$$\frac{35\mu g/mg \times 5ml}{35\,000\mu g/ml}$$
$$= 0.005ml = 5\mu L \text{ in } 5mL$$

- **Activities:**
  - Transformation of RFP
  - We prepared kanamycin stock at 35mg/µl
  - Autoclave LB liquid medium

- Pending: transfer bacteria to liquid medium

August 22, 2012

- **Members:** Elkjaer
- **Activities:**
  - Transfer bacteria (RFP) to liquid medium

August 23, 2012

- **Members:** Paul
- **Activities:**
  - We did a Mini Prep of 5 tubes with RFP. We used the protocol "Dirty Mini Prep," but we took 5ml from the pellet instead of 1.5ml.

- **Members:** Katherine & Arleny
- **Activities:**
  - Electrophoresis:

a.) Weight ladder

b.) RFP

c.) RFP

d.) RFP

e.) RFP

f.) RFP

  - Three DNA bands which belonged to the plasmid appeared, but they were not very clear.

September 1, 2012

- **Members:** Natasha
- **Activities:**
    - Glycerol stock for RFP.

    a.) 1ml glycerol at 40%

    b.) 1ml bacteria with RFP

September 11, 2012

- **Members:** Natasha
- **Activities**:
    - Genes arrived!
    - The genes were lyophilized. Therefore, before resuspending, they must be centrifuged.
    - To resuspend:
        - Resuspend lyophilized DNA in 20µL of TE Buffer (10mM Tris, 0.1mM EDTA, pH 7.5-8.0) or water to achieve an approximate stock concentration of 0.1µg/µL (100ng/µL)
        - Incubate at room temperature for 30 minutes and then vortex for 20 secs.
        - Centrifuge the tubes at 10 000xg for 1 minute.
        - Create aliquots (optional)
        - Store DNA at -20°C
        - $\frac{2\mu g}{} = 0.1\mu g/\mu L$
        - 20µL
    - Each lyophilized tube has 2µg of plasmid.
    - Following the previous protocol, we re-suspended the transcription regulator, putative promoter, cioA and cioB cyanide insensitive.

- Molecular weight of the genes: every gene is in the vector pUCIDT with Ampicillin resistance.

Genes	Molecular weight
pUCIDT (plasmid)	2800bp
Transcription regulator	1991bp
Putative promoter	329bp
cioA	1526bp
cioB	1067bp

Genes + plasmid	Molecular	Resistance
T. Regulator + pUCIDT	4 791bp	AMP
P. Promoter + pUCIDT	3 129bp	AMP
cioA + pUCIDT	4 326bp	AMP
cioB + pUCIDT	3 867bp	AMP

- What was done today:

  a.) We did a transformation of 4 genes

  b.) Autoclave liquid medium

  c.) We did 10 petri dishes with AMPICILLIN

  5 petri dishes with KANAMYCIN

  5 petri dishes with CHLORAMPHENICOL

  d.) We grew bacteria without plasmid in liquid medium to make competent cells.

September 12, 2012

- **Members:** Dafne, Paul, Andres & Emily

- **Activities:**
    - Transferred cultures to a liquid medium.

      a.) 1 falcon tube/ gen → LB + Amp (4)

      b.) 1 negative control

      c.) We transferred cells without plasmid to make them competent cells.
    - We made a stock 0.1M$CaCl_2$

      a.) 3.67g$CaCl_2$ + 250mL $H_2O_{(g.m.)}$
    - Tomorrow Thursday:

      a.) Glycerol stock for 4 genes (PP, TR, cioA and cioB)

      b.) Make competent cells

      c.) Mini Prep

      d.) Electroforesis

      $$\frac{33mg}{mL} \times 20mL = 660mg$$
    - Chloramphenicol
        - We prepared Chloramphenicol stock: 33mg/mL with 100% ethanol

September 13, 2012

- **Members:** Andres Nieves
- **Activities:**
    - Autoclaved 1L of LB medium
    - Prepared glycerol stock for bacteria/ competent cells, transcriptional regulator (TR), putative promoter (PP), cioA and cioB. We froze them at 80°C in a box that says iGEM.

- From the 1L of autoclaved LB, we took 100mL and poured it into an autoclaved bottle. Then we added 1ml of competent cells.
- We left the 1/100 dilution (mentioned before) in a shaker. We also left the tubes with genes (cioAB, cioAA, etc.)

- **Members:** Paul
- **Activities:**
  - We made competent cells using the original protocol taken from last year's wiki.
  - A total of 24 microtubes in -80°C labeled as C.C. (competent cells)

- **Members:** Orlando
- **Activities:**
  - We did a Mini Prep for the Transcriptional Regulator, P.P., cioA and cioB.
  - We also measured the concentration of each:

    a.) cioB → 264.6ng/µL

    b.) P.P. →117.5ng/µL

    c.) cioA →204.7ng/µL

    d.) TR → 120.1ng/µL

September 13, 2012

- **Members:** Tamara
  - Electrophoresis with gel at 1% of agarose.
  - We measured the concentration in the nanodrop and these were the results:

    a.) White ---0

    b.) cioB---264.6ng/µL

260/280: 2.15

260/230: 1.99

c.) PP---117.5ng/µL

260/280: 2.12

260/230: 1.25

d.) cioA---204.7ng/µL ~ 2.05ng/µL

260/280: 2.12

260/230: 1.79

e.) TR---120.1ng/µL

260/280: 2.12

260/230: 1.36

- The order in the electrophoresis was the same: cioB, P.P., cioA, T.R., the image was saved in the iGEM folder. Check it out! Bless!

September 14, 2012

- **Members:** Sergio & Orlando
- **Activities:**
    - Digestion with Xba1 and Spe1 from P.P., T.R., cioA and cioB (2 hours at 37°C). - Denaturalization for 15 minutes at 80°C.

Digestion	
Buffer 4	5µL
BSA	0.5µL
Xba1	1µL
Spe1	1µL
DNA	20µL
H2O	22.5µL
Final Volume	**50µL**

- **Members:** Katherine & Arleny
- **Activities:**
    - Real values = LB 25g/L

      Agar 15g/L
    - We prepared a medium

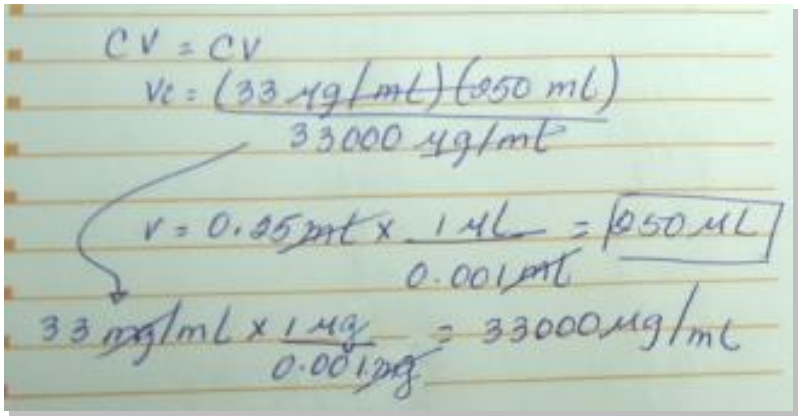
      a.) 6.25g LB for 250mL distilled water

      b.) 3.75g Agar for 250mL distilled water
    - We autoclaved tips, the medium and eppendorf.
    - Chloramphenicol:

      a.) C_i = 33mg/mL

      b.) C_f = 33µg/mL

      c.) V_f= 250mL



- We prepared 11 plates with LB + Chloramphenicol

- **Members:** Arleny, Katherine & Emily

- **Activities:**

  - Electrophoresis:

    a.) Weight Ladder

    b.) P.P.

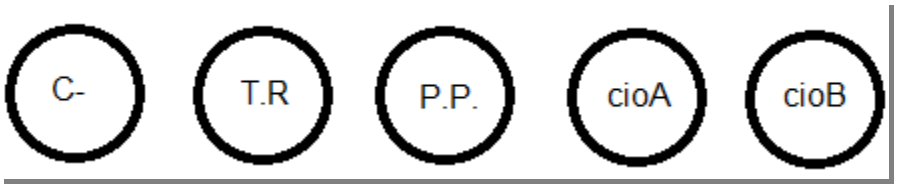
    c.) T.R.

    d.) cioA

    e.) cioB

    f.) 5µL Sample

- We made a transformation of 4 genes ("linked"): pSB1C3 using the commercial competent cells.



- Transformation with the system of RFP to try competent cells made by Paul.

September 15, 2012

- **Members:** Elkjaer & Sergio
- **Activities:**
    - We re-suspended the primer in TE:

      a.) BBa_G1001-Prefix

      100µM Stock
      35.5nmoles →355µL (100µM/µl)
      Then we took 30µL and added 270µl of TE leaving 10µL.

      b.) BBa_G100-Suffix

      100µM Stock
      27.1nmoles →271µl (100µM/µL)
      We took 30µl and added 270µl of TE(10µl)

      c.) FBBa_G00100

      100µM stock
      28.2nmoles →282µl
        - We did the same procedure

      d.) RBBa_G00101

      100µM stock
      31.8nmoles →318µl
      We did the same procedure

- **Members:** Tamara & Emily
- **Activities:**
    - We transferred the following to liquid medium:

      a.) 2.5mL LB medium

      b.) 2.5µL Chloramphenicol

      c.) Cultures with:

      cioA

cioB
P.P.
d.) We also made a C-

- We left bacteria with RT growing in a petri dish with LB + Chlorampenicol

- **Members:** Paul
- **Activities:**
  - PCR of the following parts:

1A (PP)		
Degrees	**Time**	**Cycle**
94°C	5:00 minutes	
94°C	30 Secons	35 cycle
58.5°C	1:00 minute	
72°C	30 Seconds	
72°C	10 minutes	
4°C	∞	

2A (PP)		
Degrees	**Time**	**Cycle**
94°C	5:00 minutes	
94°C	30 Seconds	35 cycle
53°C	1:00 minute	
72°C	30 Seconds	
72°C	10 minutes	
4°C	∞	

1B (RT)		
Degrees	**Time**	**Cycle**
94°C	5:00 minutes	
94°C	30 Seconds	35 cycle
58.5°C	1:00 minute	
72°C	1:00 minute	
72°C	10 minutes	
4°C	∞	

2B (RT)		
Degrees	**Time**	**Cycle**
94°C	5:00 minutes	
94°C	30 Seconds	
53°C	1:00 minute	35 cycle
72°C	1:00 minute	
72°C	10 minutes	
4°C	∞	

2B (RT)		
Degrees	**Time**	**Cycle**
94°C	5:00 minutes	

94°C	30 Seconds	
53°C	1:00 minute	35 cycle
72°C	1:00 minute	
72°C	10 minutes	
4°C	∞	

1C (cioA)		
Degrees	**Time**	**Cycle**
94°C	5.00 minutes	
94°C	30 seconds	**35 cycles**
58.5°C	1.00 minute	
72°C	1.00 minute	
72°C	10 minutes	
4°C	∞	

2C (cioA)		
Degrees	**Time**	**Cycle**
94°C	5.00 minutes	
94°C	30 seconds	**35 cycles**
58.5°C	1.00 minute	
72°C	1.00 minute	

72°C	10 minutes
4°C	∞

- The tubes with #1 have the primers BBa_G1000 and BBa_G1001

- **Members:** Paul & Marla
- **Activities:**
  - The tubes with #2 have the primers FBBa_G00100 and RBBa_G00101
  - We also did a tetracycline stock at 50mg/mL.

September 16, 2012

- **Members:** Dafne & Orland
- **Activities:**
  - We measured in nanodrop 1µL of TE as blank
  - Results:

    a.) Blank : -0.9ng/µL

    b.) RFP2: 1342ng/µL

    c.) RFP1: 1453.3ng/µL

    d.) GFP1: 59.2ng/µL

    e.) GFP2: 19.0ng/µL

    f.) RBS1: 46.7ng/µL

    g.) RBS2: 17.6ng/µL

    h.) Ter1: 13.6ng/µL

    i.) Ter2: 14.4ng/µL

    j.) T7: 65.9ng/µL

*It is not recommended to digest with a small [ ].

- Orlando did a Mini Prep of PP, cioA, cioB. (BB) with pSB1C3.
- They were labeled as “A,” “B” and “PP.” They were left in the pre-PCR refrigerator.
- The Mini Prep done by Orlando was measured in nanodrop:

  a.) C-: 2.5ng/µL

  b.) P.P.: 545ng/µL

  c.) cioB: 491.8ng/µL

  d.) cioA: 554ng/µL

- We did a digestion of:

Substance	cioA	cioB
Buffer 4 (green)	5µL	**5µL**
Red Buffer	-	**5µL**
BSA	0.5µL	**0.5µL**
E}nzyme Xba1	-	**1µL**
Pst1	-	**1µL**
Spe1	1µL	**-**
EcoR1	1µL	**-**
DNA	10µL	**10µL**
H_2O	32.5µL	**27.5µL**
Final Volume	**50µL**	**50µL**

*Remember: cioA=204.7ng/mL

cioB= 264,6ng/mL

- We also did a digestion of the Mini Prep done by Orlando (labeled as “A,” “B” and “P.P”):

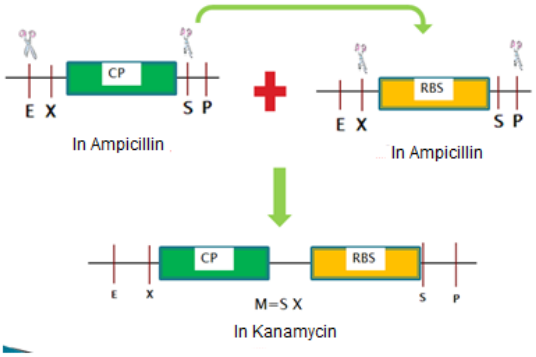
Substance	P.P	cioA	cioB
Buffer green	5uL	5uL	5uL
Red Buffer	__	__	5uL
BSA	0.5uL	0.5uL	0.5uL
Eco R1	1uL	1uL	__
Spe1	__	1uL	__
Pst1	__	__	1uL
Xba1	__	__	1uL
DNA	10uL	10uL	10uL
H_2O	32.5uL	32.5uL	27uL
Final Volume	50uL	50uL	50uL

*See [ ] in nanodrop (previous page)

- We transferred Ter, GFP, RBS and T7 to a liquid medium (they were in a glycerol stock) and we left them incubating in a shaker at 37°C.
- We also did one digestion to try some enzymes (we don't know if they work or not) that we found at the -80°C. They were labeled as "E," "X," "P," and "S."
- Everything that we digested were left in the thermocycler at 37°C for 2 hours.

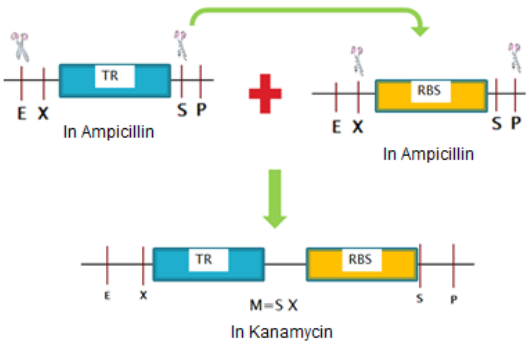
*Scheme of the first digestions:

1. Constitutive Promotor with RBS



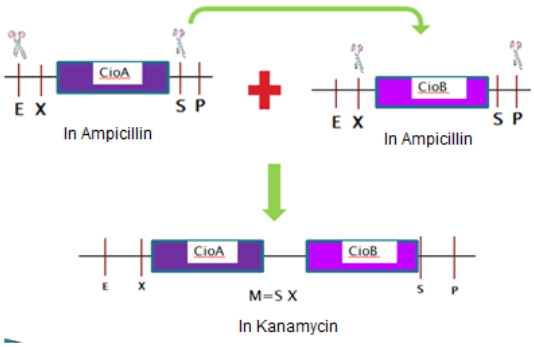
Kanamycin plasmid must be digested with E and P.

2. Trascriptional Regulator with RBS



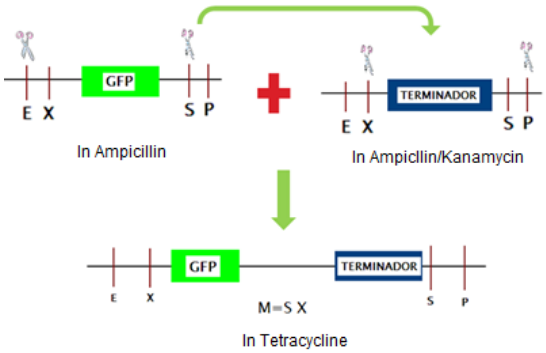
Kanamycin plasmid must be digested with E and P

3. CioA with CioB



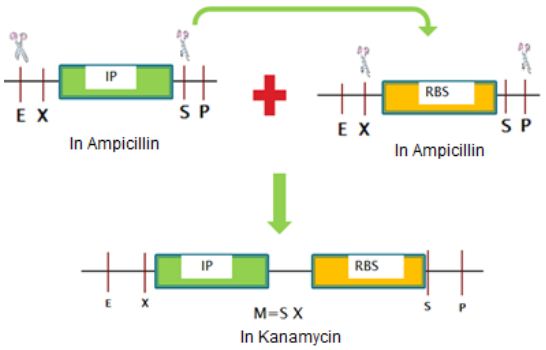
Kanamycin plasmid must be digested with E and P

4. Reporter1 + Terminador



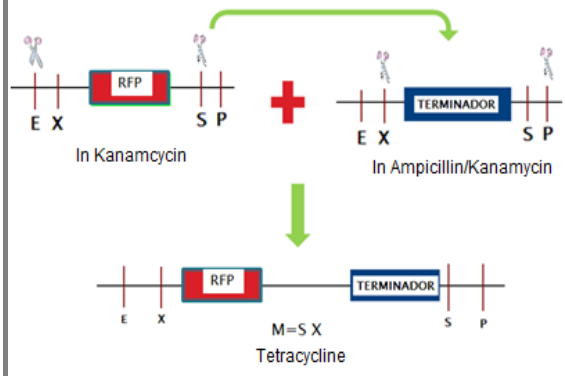
Kanamycin plasmid must be digested with E and P

5. Inducible Promoter with RBS



Kanamycin plasmid must be digested with E and P

6. Reporter2 + Terminator



Kanamycin plasmid must be digested with E and P

- We did a ligation of cioA and cioB in plasmid pSB1K3.

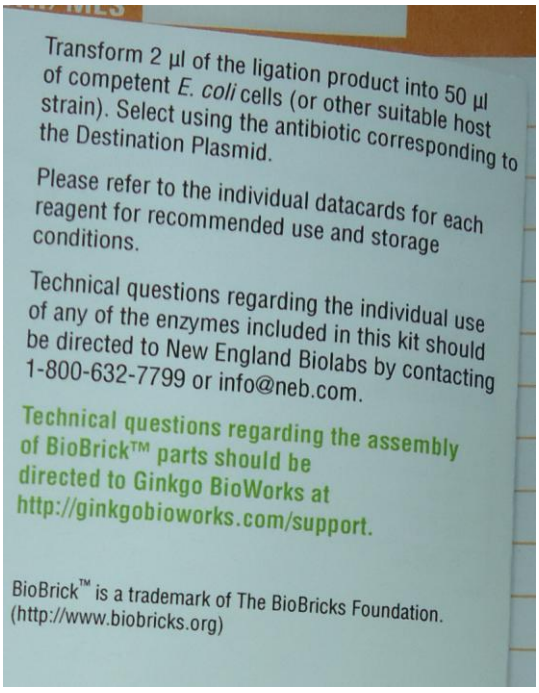
Substance	Quantity
H_2O	**12.5µL**
T4 Buffer	**2.5µL**
K vector	**1.0µL**
cioA	**4.0µL**
cioB	**4.0µL**
T4 Ligase	**1µL**
Final Volume	**25µL**

- We left it overnight in the 4°C refrigerator (virology room). It has a pink tube rack and the tubes are labeled as CA and CB.

Members: Paul

- Electrophoresis of the PCR (from yesterday) iGEM 2012, labeled as “standardization of PCR Sept 16, 2012.”

- We also left growing the P.P., TR, cioA and cioB from the glycerol stocks (Sept 13, 2012) to make more Mini Prep.



- Mini Prep of all the falcon tubes (50ml)
- Linearization with EcoR1 (Use buffer 4)
- Electrophoresis
- Send pictures of the electrophoresis and analyze them.
- If the weighs are right, measure the concentration of DNA in nanodrop.
- Then follow protocol of Digestion/Ligation
- Transformation

Remember to digest the plasmid

September 17, 2012

- **Members:** Natasha & Paul
- **Activities:**
  - We linearized with EcoR1 all the Mini Preps from June 15, and T7 from June 22; also cioA, cioB, TR and PPi from September 13. (Including RFP1 that Paul did).
  - Total: 11 linearizations.
  - Reaction:

(100ng) DNA	X µL	
H_2O	**18.5 up to 50µL**	
3 Buffer	5µL	**6.5**
BSA	0.5µL	
EcoR1	**1.0µL**	

  - Amount of µL of DNA that should be used in each gene in order to have approximately a concentration of 100ng:

Gene	Quantity	Water
RFP 1	0.5µL to **0.2µL**	**43.3µL**
GFP 1	2µL	**41.5µL**
GFP 2	5.5µL	**38µL**
RBS 1	2.5µL	**41µL**
RBS 2	6.0µL	**37.5µL**
***Ter 1**	7.5µL	**36µL**
Ter 2	7.5µL	**36µL**
T7	2.0µL	**41.5µL**
PPi	1.0µL	**42.5µL**
TR	1.0µL	**42.5µL**
cioA	0.5µL	**43µL**
cioB	**0.5µL**	**43µL**

*Ter 1 didn't have enough DNA from the Mini Prep, we didn't reach a µL.

- Concentrations of the products from the Mini Prep:

Gene	Quantity
T7	**3 055.1ng/µL**
cioA	**1 902.1ng/µL**
Ter	**1 639.7ng/µL**
cioB	**1 723.2ng/µL**
TR	**562ng/µL**
RBS	**2 275.9ng/µL**
PP	**380ng/µL**
GFP	**2 572ng/µL**

- **Members:** Marla & Emily
- **Activities:**
    - We did LB medium (autoclave later)

      a.) 25g in 1 000mL distilled water
    - We did LB + Agar (autoclave later)

    a.) 14.5g Agar

    b.) 750mL LB
    - Electrophoresis: (1µL of Buffer in each)

Lane	Quantity
Weight Ladder	**2µL**
RFP 1	**3µL**
GFP 1	**3µL**
GFP 2	**3µL**
RBS 1	**3µL**
RBS 2	**3µL**
T1	**3µL**
T2	**3µL**
T7	**3µL**
PPi	**3µL**
TR	**3µL**
cioA	**3µL**
cioB	**3µL**

- We prepared petri dishes with the autoclaved LB + Agar:

a.) 10 plates with Kanamycin (275mL)

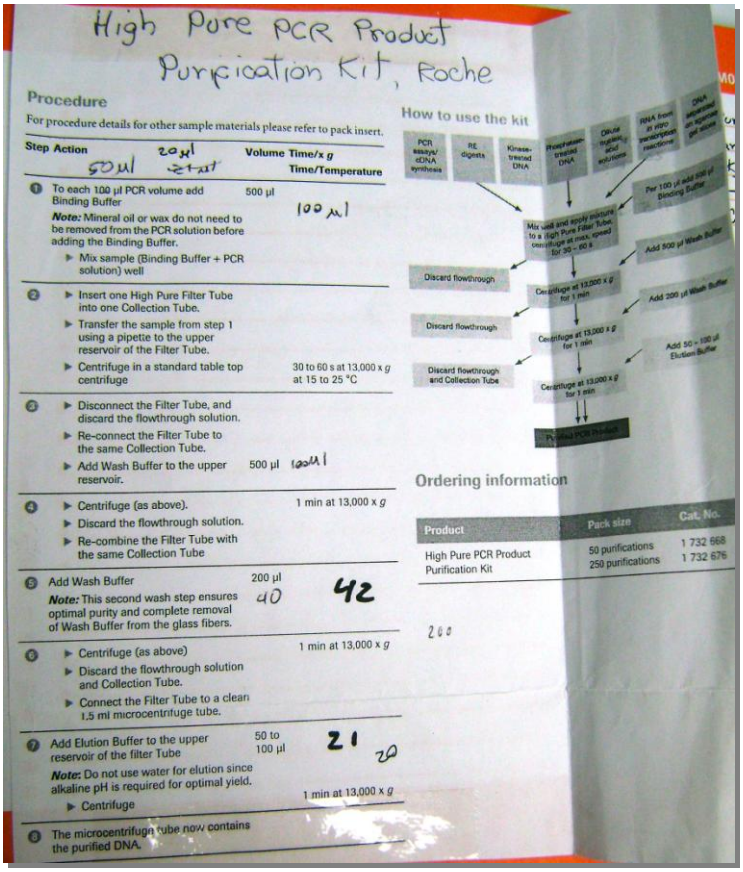
b.) 6 plates with Ampicillin (150mL)

c.) 6 plates with Tetracycline (150mL)

d.) 6 plates with Chloramphenicol (150mL)

September 18, 2012

- **Members:** Natasha
- **Activities:**
    - Purification of the Mini Prep done by Paul (Sept. 17) using the kit, “High Pure PCR Purification Kit,” Roche.



    - Concentrations of the purifications:

Part	Concentration
Ter	**41.1ng/µL**
CioB	**48.8ng/µL**
CioA	**43.0ng/µL**
GFP	**57.9ng/µL**
RBS	**35.5ng/µL**
TR	**29.3ng/µL**
P.P.	**34.3ng/µL**
T7	**43.5ng/µL**
Mean Value	**41.5ng/µL**

- In order to linearize, we must use the following buffers for each enzyme:

Enzyme	Buffer
EcoR1-HF	**4**
Xba1	**4**
Spe1	**4**
Pst1	**3**

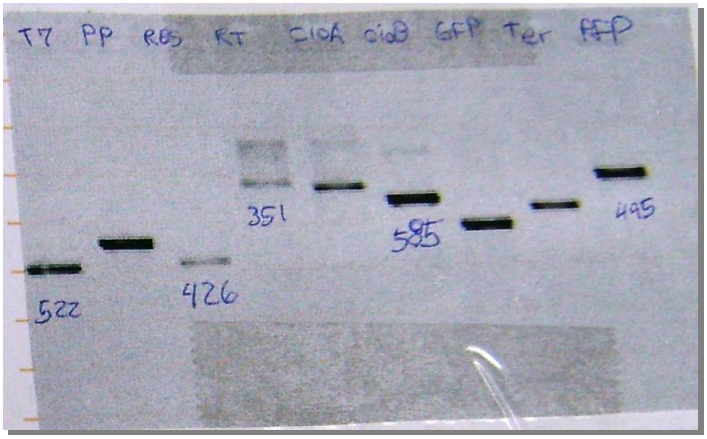
- To make ligations with the protocol Briobrick, use buffer 2.
- I did a digestion of the purified Mini Prep again. I used 12µL per sample to reach a concentration of 500ng of DNA approximately. I calculated the mean value of all the concentrations to use a standard volume of DNA and make a master mix of the reaction.

Reaction	Volume µL	X 9	
H_2O	31.5	283.5µL	**Master Mix**
Buffer 4	5.0	45µL	
BSA	0.5	4.5µL	
EcoR1-HF	1.0	9µL	
DNA	**12**		

- RFP = 22.5ng/µL
- We also did a digestion of the RFP:

Reaction	Volume
H2O	**21.5µL**
Buffer 4	**5µL**
BSA	**0.5µL**
EcoR1	**1µL**
DNA	**22µL**

- **Members:** Tamara
- **Activities:**
  - Electrophoresis



- Digestion of the following parts:

Parts	Plasmid
cioA + cioB	**Kanamycin**
C.P. + RBS	**Kanamycin**
TR + RBS	**Kanamycin**
P.P. + RBS	**Kanamycin**
GFP + Ter	**Tetracycline**
RFP + Ter	**Tetracycline**

- Reaction of the digestion:

  a.) cioA, CP, TR, PP, GFP, RFP → cut with E and S

  b.) cioB, RBS, Ter → cut with X and P

  c.) Plasmids pSB1K3 and pSB1T3 → cut with E and P

- Final volume = 25µL

First Reaction		
Substance	**Volume**	**x 6 Master Mix**
H_2O	12.25µL	**73.5µL**
Buffer 2	2.5µL	**15µL**
BSA	0.25µL	**1.5µL**
EcoR1-HF	1.0µL	**6µL**
Spe1	1.0µL	**6µL**
DNA	**8.0µL**	

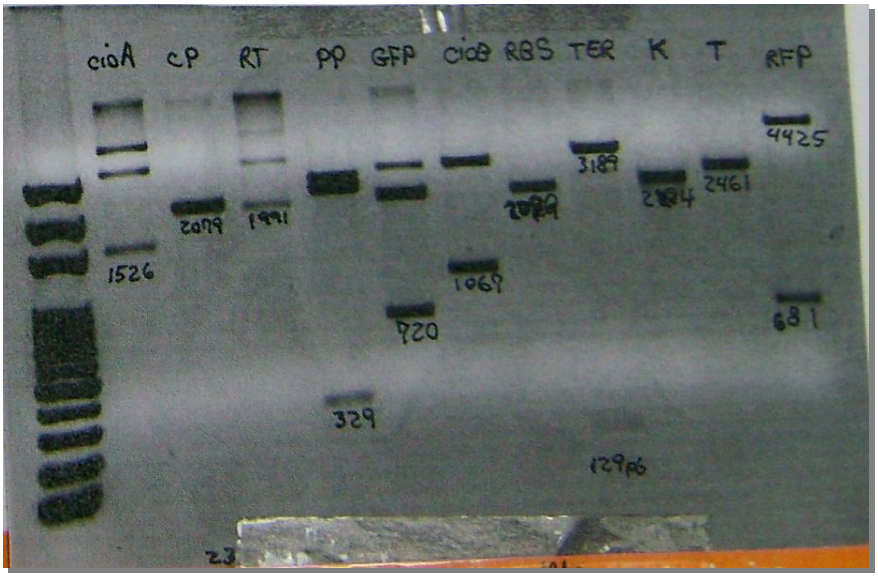
Second Reaction		
Substance	**Volume**	**x 4 Master Mix**
H_2O	12.25µL	**50µL**
Buffer 2	2.5µL	**10µL**
BSA	0.25µL	**1µL**
Xba1	1.0µL	**4µL**
Pst1	1.0µL	**4µL**
DNA	**8.0µL**	

Third Reaction		
Substance	**Volume**	**x 3 Master Mix**
H_2O	16.95µL	**50.85µL**
Buffer 2	2.5µL	**7.5L**
BSA	0.25µL	**0.75µL**

EcoR1	1.0µL	**3µL**
Pst1	1.0µL	**3µL**
DNA	**3.3µL**	

- Electrophoresis of the digestion: OK!

RFP	
Substance	**Volume**
H_2O	**9.25µL**
Buffer 2	**2.5µL**
BSA	**0.25µL**
EcoR1	**1µL**
Spe1	**1µL**
DNA	**11µL**

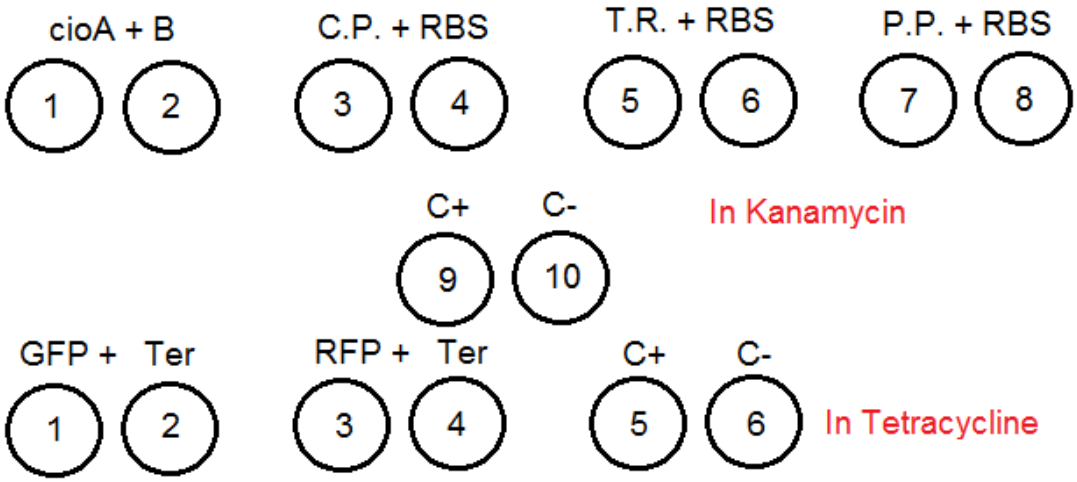


- Ligation of the new BBs:

Substance	Volume
Ups Part	**2µL**
Downs Part	**2µL**
Plasmid	**2µL**
Buffer	**2µL**
Ligase	**1µL**
H2O	**11µL**
Final Volume	**20µL**

September 19, 2012

- **Members:** Natasha, Dafne & Andres N.
- **Activities:**
  - Transformation of the ligations (duplicate), 1 positive control, 1 negative control using plasmids with antibiotics.



- Possible:
  - C+ : 20H → Plate 3 Reporter
  - BBa_K274003 V10 Operon ABDE
  - 2µL Ligations
  - 50µL of Competent cells “C.C.”

September 20, 2012

- **Members:** Sergio
- **Activities:**

C_i= 35mg/mL V_i=?

C_f = 35µL/mL V_f = 2mL

Kanamycin stock at 35mg/mL

$$C_i V_i = C_f V_f$$

(35mg/mL) (Vi) = (35µL/mL) (2mL)

- We prepared 14 tubes of 50mL with 2mL of LB-Kanamycin.
- We made cultures of the following transformations:

  a.) cioA + B (1)

  b.) cioA + B (2)

  c.) P.P. + RBS (1)

  d.) P.P. + RBS (2)

  e.) CP + RBS

  f.) T.R. + RBS

  g.) C+

h.) C-

- WAIT until they grow to make Mini Prep
- The transformations made in LB-T haven't grown yet. (20/9/2012- Time: 11:00AM)
- One of the ligation plates CP + RBS in LB-Kan hasn't grown yet.
- One of the plates TR + RBS in LB-Kan has little growth. Must wait before doing culture in LB-Kan liquid medium (20/9/2012- Time: 11:00AM)

- **Members:** Miguel, Elkjaer & Emily
- **Activitites:**
  - We prepared ethanol 70% and 50%.
  - We prepared tetracycline with the ethanol 50%.
    - 0.075g in 5mL ethanol
  - We also prepared LB + Agar plates (we autoclaved the LB + Agar medium in 4 different Erlenmeyer).

Amount of Plates	Antibiotic	LB	Agar	Amount of Antibiotic	Concentration
10	Tetracycline	77mL	1.155g	77µL	**15mg/mL**
10	Kanamycin	77mL	1.155g	77µL	**35mg/mL**
5	Chloramphenicol	42mL	0.639g	42µL	**33mg/mL**
5	Ampicillin	42mL	0.639g	42µL	**50mg/mL**
Total of 30 plates → 3.57g Agar → 238mL LB					

- **Members:** Paul & Natasha
- **Activities:**
    - Paul did a Mini Prep from yesterday's ligations and then we purified them.
    - Concentrations:

Parts	Concentrations	µL
(250ng/µL) cioAB 1	71ng/µL	3.5
(250ng) cioAB 2	34.8ng/µL	7.2
C.P. + RBS	10.5ng/µL	9
T.R. + RBS	4.2ng/µL	9
P.P. + RBS	8.0ng/µL	9
T.R. + RBS 2	**285ng/µL**	**0.90**

    - Reactions from linearization

cioAB 1	
H_2O	**17.75**
Buffer 4	**2.5**
BSA	**0.25**
EcoR1	**1.00**
DNA	**3.50 (250ng)**
Final Volume	**25µL**

TR + RBS 2	
H_2O	**20.35**
Buffer 4	**2.5**
BSA	**0.25**
EcoR1	**1.00**
DNA	**0.90 (250ng)**
Final Volume	**25µL**

cioAB2	
H_2O	14.05
Buffer 4	2.5
BSA	0.25
EcoR1	1.00
DNA	7.2
Final Volume	25uL

CP+RBS	
H_2O	0.5
Buffer 4	1
BSA	0.2
EcoR1	0.5
DNA	8
Final Volume	10uL

T.R. + RBS	
H_2O	0.5
Buffer 4	1
BSA	0.2
EcoR1	0.5
DNA	8
Final Volume	10µL

P.P. + RBS	
H_2O	0.5
Buffer 4	1
BSA	0.2
EcoR1	0.5
DNA	8
Final Volume	10µL

- **Members:** Elkjaer & Emily
- **Activities:**
    - We transferred the following parts to different plates with tetracycline:

      a.) GFP + Ter

      b.) RFP + Ter 2

      c.) C+

      d.) RFP + Ter

      e.) C-

- We transferred the following parts to different plates with Kanamycin:

  a.) P.P. + RBS

  b.) C-

September 21, 2012

- **Members:** Dafne & Orlando
- **Activities:**
  - Transformations of: (*purified* → 2µLDNA and 50µL Competent Cells)

    a.) cioAB1

    b.) cioAB2

    c.) TR + RBS

    d.) TR + RBS 2

    e.) C.P. + RBS

    f.) P.P. + RBS

  - Deborah and Carolina gave us 5 plates with tetracycline. *wrapped in aluminum foil."

    12µg/mL ethanol

  - So, we transformed with the ligations: RFP and GFP in tetracycline too.

*Remember that the transformations have cells, so they must be saved at 4°C not at -20°C because they will die.

- The transformations were left in the virology room at 4°C (blue test tube rack)
- There are 7 Kanamycin plates and 3 tetracycline plates in the Pre-PCR incubator.

Kanamycin	Tetracycline
cioAB1	GFP + Ter
cioAB2	RFP + Ter
TR + RBS 1	C-
TR + RBS 2	
C.P.+ RBS	
P.P. + RBS	
C-	

- Marla transferred TR + RBS colonies (1,2 and 3) to a liquid medium.

September 22, 2012

- **Members:** Katherine & Arleny
- **Activities:**
  - We transferred to liquid medium the cultures from the following plates:

    *LB (2.5mL) + Kanamycin (2.5µL)

    (2 falcon tubes each)

    a.) cioAB 2

    b.) CP + RBS

    c.) TR + RBS2

    d.) PP + RBS

    e.) C-
- **Members:** Orlando
- **Activities:**
  - Polyacrylamide gel

- **Members:** Katherine & Arleny
- **Activities:**
    - Electrophoresis of the digestion:

      a.) Weight Ladder

      b.) P.P.

      c.) TR

      d.) cioA

      e.) cioB

      f.) cioB
- **Members:** Tamara & Orlando
    - Mini Prep of the cultures that were transformed with Chloramphenicol plasmids.
    - Concentrations (nanodrop)

Part	Concentration	260/280	260/230
Blank	-0.4		
cioA	1 289.8ng/µL	2.08	2.07
cioA1	3 471.7ng/µL	1.89	1.80
cioB	2 814.7ng/µL	1.83	1.70
cioB1	524.4ng/µL	2.01	1.76
P.P.	821.9ng/µL		
P.P.1	1 969.6ng/µL	2.09	2.02
Blank	**-0.2**		

- **Members:** Orlando
- **Activities:**

- Linearization with EcoR1

- **Members:** Tamara
- **Activities:**
  - I transferred the following cultures to a liquid medium:

    1.) (neg)

    2.) RFP + Ter (1)

    3.) RFP + Ter (2)

    4.) GFP + Ter (1)

    5.) GFP + Ter (2)

  - Marla added antibiotic.
- **Members:** Miguel & Paul
- **Activities:**
  - Digestion

Digestion	Ligation
4mL	**5mL**
10mL	**6mL**
5mL	**2mL**
5mL	**4.5mL**
5mL	**3mL**

a.) Pt = 6 + 5 + 1 + 2 - 6

b.) P.P. = 2 + 5 + 1 +2 – 10

c.) cioA = 4.5 + 5 + 1 + 2 – 7.5

d.) cioB = 3 + 5 + 1 + 2 – 9

- I made digestion of:

  a.) RBS 1 = 46.7ng/µL

  b.) Ter 2 = 14.4ng/µL

  c.) T7 = 65.9ng/µL

  d.) C.P. + RBS = 10.5ng/µL

CP + RBS	
H_2O	**3.3µL**
Buffer 2	**1.5µL**
BSA	**0.2µL**
EcoR1	**0.5µL**
Pst1	**0.5µL**
DNA	**9µL**
Final Volume	**15µL**

Ter	
H_2O	**2.3µL**
Buffer 2	**1.5µL**
BSA	**0.2µL**
EcoR1	**0.5µL**
Pst1	**0.5µL**
DNA	**10µL**
Final Volume	**15µL**

RBS1	
H_2O	8.3uL
Buffer 2	1.5uL
BSA	0.2uL
EcoR1	0.5uL
Pst1	0.5uL
DNA	4.0uL
Final Volume	15uL

T7	
H_2O	8.3µL
Buffer 2	1.5µL
BSA	0.2µL
EcoR1	0.5µL
Pst1	0.5µL
DNA	4µL
Final Volume	15µL

- **Members:** Tamara & Arleny
- **Activities:**
    - Electrophoresis in the following order:

    a.) Weight Ladder

    b.) cioA

    c.) cioA 1

d.) cioB

e.) cioB1

f.) P.P.

g.) P.P. 1

- **FINISHED ONE BIOBRICK!**
- **Members:** Tamara, Arleny & Elkjaer
- **Activities:**
    - Transformation
    - Inventory (plates): 23/09/2012 – 3:30a.m.

Antibiotic	Petri Dish (Big)	Petri Dish (Small)
Chloramphenicol	9	----
Ampicillin	----	22
Kanamycin	----	10
Tetracycline	**3**	**----**

- **Members:** Dafne
- **Activities:**
    - I did 24 plates LB + Chloramphenicol (small one)

$$\frac{15g \times 250}{1\,000} = 3.75g \text{ Agar}$$

September 23, 2012

- **Members:** Elkjaer

- **Activities:**
    - Mini Prep of:

Part	Concentration	260/280	260/230
GFP + Ter 1	307.6ng/μL	1.73	**1.52**
GFP + Ter 2	306.5ng/μL	1.63	**1.26**
CP + RBS	6 245.2ng/μL	2.01	**2.03**
cioA + B 1	3 743.4ng/μL	1.84	**2.06**
P.P. + RBS	919.2ng/μL	2.13	**2.14**
TR + RBS 2	318.2ng/μL	2.08	2.09

    - Purified tube 1 and 2

      a.) GFP + Ter = 8.2ng/μL 260/280 = 2.79

      b.) GFP + Ter = 12.0ng/μL 260/280 = 4.38

      - Agarose gel:

      a.) Agar 0.7g/100mL TAE 1X

      b.) 2μL Ethidium Bromide
    - Digestion of:

      a.) Upstream parts: CP + RBS, cioAB

      b.) Downstream parts: TR + RBS, GFP + Ter, cioAB
    - Passed to pSB1C3 parts: CP + RBS, TR + RBS, cioA+B, P.P. + RBS.
    - Upstream digestions:

CP + RBS	
H_2O	16uL
Buffer 2	2.5uL
BSA	0.5uL
Eco R1	1uL
Spe1	1uL
DNA	4uL
Final Volume	25uL

CioAB	
H_2O	13.3µL
Buffer 2	2.5µL
BSA	0.5µL
Eco R1	1µL
Spe1	1µL
DNA	6.7µL
Final Volume	25µL

- Downstream digestions:

GFP + Ter	
H_2O	1µL
Buffer 2	2.5µL
BSA	0.5µL
Xba1	1µL
Pst1	1µL
DNA	20µL
Final Volume	25µL

:

TR + RBS	
H_2O	12µL
Buffer 2	2.5µL
BSA	0.5µL
Xba1	1µL
Pst1	1µL
DNA	8µL
Final Volume	**25µL**

cioAB	
H_2O	**13.3µL**
Buffer 2	**2.5µL**
BSA	**0.5Ml**

Xba1	**1µL**
Pst1	**1µL**
DNA	**6.7µL**
Final Volume	**25µL**

CP + RBS	
H_2O	16µL
Buffer 2	2.5µL
BSA	0.5µL
EcoR1	1µL
Pst1	1µL
DNA	4µL
Final Volume	25µL

TR + RBS	
H_2O	12µL
Buffer 2	2.5µL
BSA	0.5µL
EcoR1	1µL
Pst1	1µL
DNA	8µL
Final Volume	25µL
CioAB	

H_2O	13.3uL
Buffer 2	2.5µL
BSA	0.5µL
EcoR1	1µL
Pst1	1µL
DNA	6.7µL
Final Volume	25µL

- Plasmids

P.P. + RBS	
H_2O	**17µL**
Buffer 2	**2.5µL**
BSA	**0.5µL**
EcoR1	**1µL**
Pst1	**1µL**
DNA	**3µL**
Final Volume	**25µL**

Tetracycline	
H_2O	**16.95µL**
Buffer 2	**2.5µL**
BSA	**0.5µL**
EcoR1	**1µL**
Pst1	**1µL**
DNA	**3.3µL**
Final Volume	**25µL**

AMP	
H_2O	16.95µL
Buffer 2	2.5µL
BSA	0.5µL
EcoR1	1µL
Pst1	1µL

DNA	3.3µL
Final Volume	25µL

Chloramphenicol	
H_2O	16.95µL
Buffer 2	2.5µL
BSA	0.5µL
EcoR1	1µL
Pst1	1µL
DNA	3.3µL
Final Volume	25µL

- In that same order we did an electrophoresis.

a.) 1µL Weight Ladder

b.) 1µL L buffer

c.) 5µL Sample

- The electrophoresis just showed the plasmids Tetracycline and Chloramphenicol; the rest didn't work.

- Since one part of the Mini Prep wasn't done properly, we transferred again cultures labeled as 1 and 2 from RFP + Ter (in tetracycline) and GFP + Ter (in tetracycline) to falcon tubes (purple, 50mL). They were left in the shaker at 37°C.

CP + RBS 1µL 15ml

x 30mL

2µL →30mL

cioAB 1µL 10mL

x 30mL

3µL → 30mL

- Nanodrop:

3’	573ng/µL
4	152.4ng/µL
3	5 051.0ng/µL
5’	1 416.2ng/µL
5	3 712.5ng/µL
6	884.6ng/µL
4’	27ng/µL
3*	103.5ng/µL
4*	4.5ng/µL
5*	66.9ng/µL
6*	72.0ng/µL

- Since the concentrations were very random, we decided that it was better to postpone the ligation and only re-suspend for today.

- Arleny and Katherine transferred cultures from the transformation TR in Chloramphenicol and P.P in Chloramphenicol to LB (2mL).

- Steps to follow on Monday 24, 7:00am

a.) Mini Prep of the following:

P.P.- C	They were left in the

TR - C	shaker
GFP + Ter –T (1 and 2)	They were left at 4°C (Hall)
RFP + Ter – T (1 and 2)	
C.P. + RBS –K	They were left in the shaker
TR + RBS – K	
cioA + B – K	
P.P. + RBS – K	

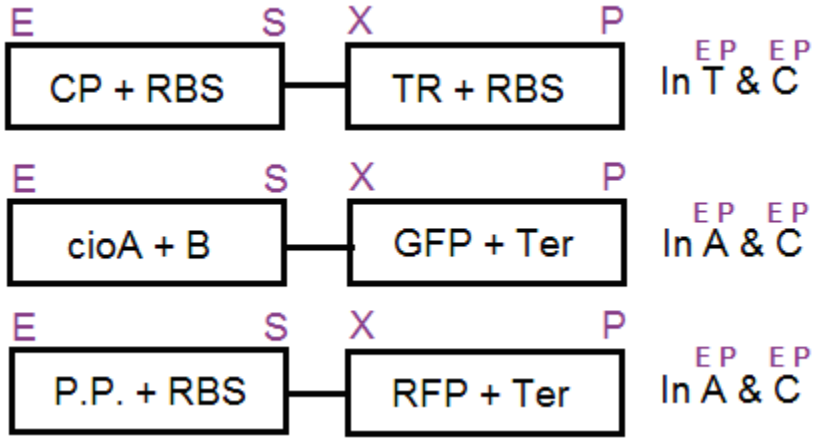
b.) Nanodrop of all the products from Mini Prep

c.) Purify: with Roche Kit…Protocol. Look for 18/9/2012

d.) Nanodrop of everything that was purified

e.) Digestion of:

- P.P – C and TR – C, to prove that we have Chloramphenicol-insert.



- Remember to digest plasmids T, A and C.

- Plasmid –vs- insert → relation of 1:3

- Final volume of digestions: 25µL/ each

f.) Electrophoresis

g.) Ligation

h.) Transformation

- Plates → A – 3

→ T – 2

→ C – 6

* Remember to transform P.P. – C and TR – C.

September 24, 2012

Members: Orlando & Emily

- Nanodrop:

Part	Concentration	260/280	260/230
TR 1	386.5ng/µL	1.94	2.10
TR 2	1 115.4ng/µL	1.85	2.07
C.P. + RBS	1 605.6ng/µL	1.83	2.09
PP1	1 106.2ng/µL	1.98	2.22
PP2	567.4ng/µL	1.98	2.04
cioAB	404.2ng/µL	2.02	2.08
PP + RBS	145.5ng/µL	2.05	1.86
TR + RBS	293.4ng/µL	2.02	2.13
RFP + Ter 1	327.8ng/µL	2.04	1.96
GFP + Ter 2	192.6ng/µL	2.08	1.96
RFP + Ter 2	236.5ng/µL	2.11	2.06
GFP + Ter 1	268.3ng/µL	2.12	2.08

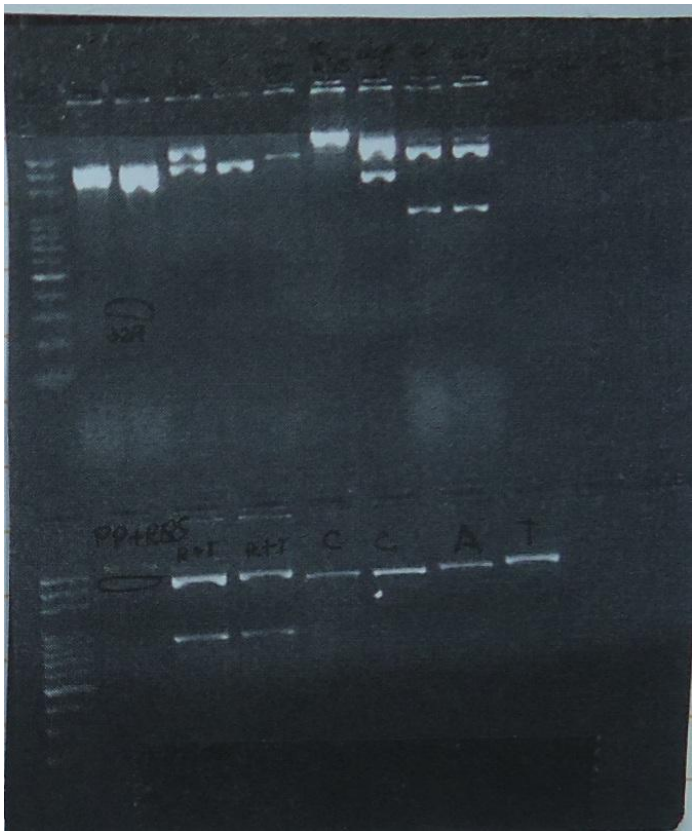
- Electrophoresis:

For the gel we added:

a.) 1.40g Agar

b.) 70mL TBE 1X

c.) 2µL Ethidium Bromide



<u>Members:</u> Natasha, Arleny & Miguel

- I did a digestion of:

a.) CP + RBS	2µL	CP + RBS
b.) TR + RBS	2µL	TR + RBS
c.) Ter	2µL	Chloramphenicol
d.) Ligase Buffer	2µL	Buffer
e.) Ligase	1µL	Ligase

f.) H_2O 11µL H_2O

- Master Mix: 77µL H_2O

14µL Buffer

7µL Ligase

September 25, 2012

Members: Arleny, Natasha & Miguel

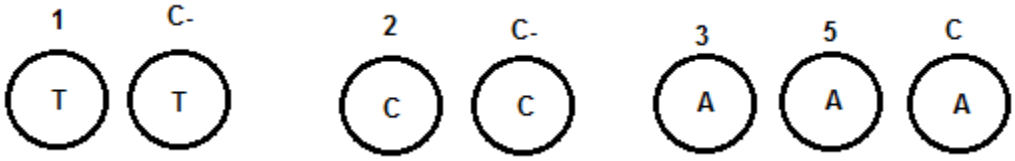
- Arleny did a transformation of the ligations:

1.) CP + RBS – TR + RBS in Tetracycline

2.) CP + RBS – TR + RBS in Chloramphenicol

3.) cioA + B – GFP + Ter in Ampicillin

5.) PP + RBS – RFP + Ter in Ampicillin



Tomorrow:

- Liquid medium of the plates from 25/9/12

- Mini Prep of pSB1C3 that Paul transformed in liquid medium (shaker).

- Find the 4 Biobricks that will be sent. Ask Orlando.

- Before sending them, we must take 2µL of the Mini Prep to make 1 transformation and that way we can have all the parts in Chloramphenicol.

Members: Marla and Lizzi

- Second digestion with EcoR1 of the ligations prepared by Natasha and Orlando.

First Group (Blue)	
DNA	1µL
EcoR1	1µL
Buffer	5µL
BSA	0.5µL
H_2O	42.5µL

Second Group (Green)	
DNA	3µL
EcoR1	1µL
Buffer	5µL
BSA	0.5µL
H_2O	40.5µL

- cioB 2µLDNA + 41.5µL

- P.P 2µL DNA + 41.5µL

Members: Lizzi & Tamara

- Electrophoresis of the second digestion of the ligation

a.) 2µL of Molecular Weight

b.) cioA1

c.) cioA2

d.) cioB1

e.) cioB2

f.) cioAB

g.) PP1

h.) PP2

i.) TR1

j.) TR2

k.) x PP1

l.) x PP2