Index:

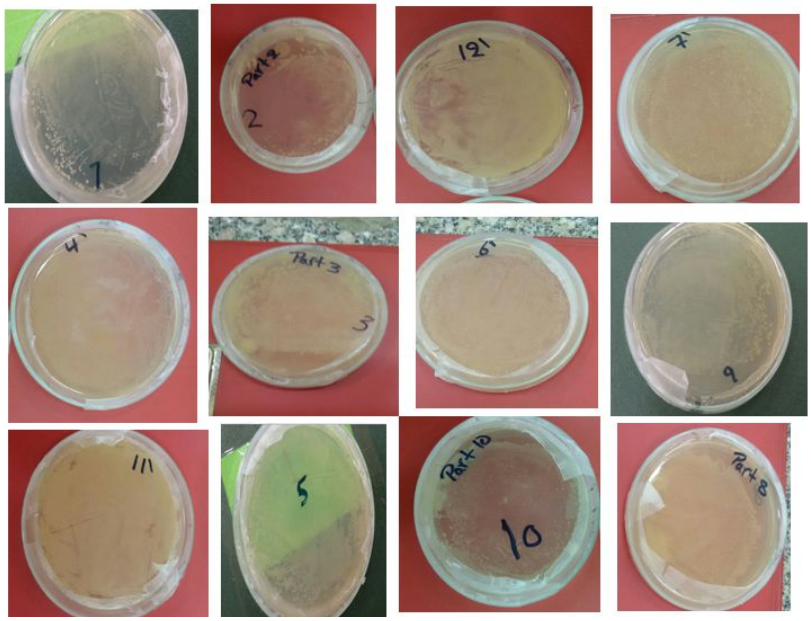
CHLORAMPHENICOL PARTS :

Part Number		
C1	BBa_K546003	Left promoter + RBS +LUXR + Double Terminator
C2	BBa_K880005	Strong Promoter + RBS
C3	BBa_R0063	Left Promoter
C4	BBa_C0062	LUXR
C5	BBa_B0015	Double Terminator
C6	BBa_R0062	Right Promoter
C7	BBa_C0061	LUXI
C8	BBa_J23108	Promoter 1
C9	BBa_J23109	Promoter 2
C10	BBa_J23111	Promoter 3
C11	BBa_I20270	GFP Device
C12	BBa_R0040	PTETR

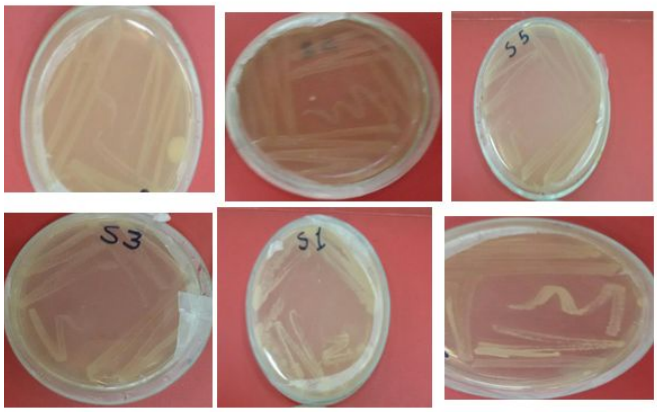
AMPICILLIN PARTS

A1	BBa_C0261	RBS+LuxI
A2	BBa_I13401	GFP+Double terminator
A3	BBa_B0034	RBS
A4	BBa_E0040	GFP
A5	Bba_E0240	RBS+GFP+Terminator
A6	Bba_I13504	GFPGENERATOR

Transforming parts from the registry :



Streaking Colonies :

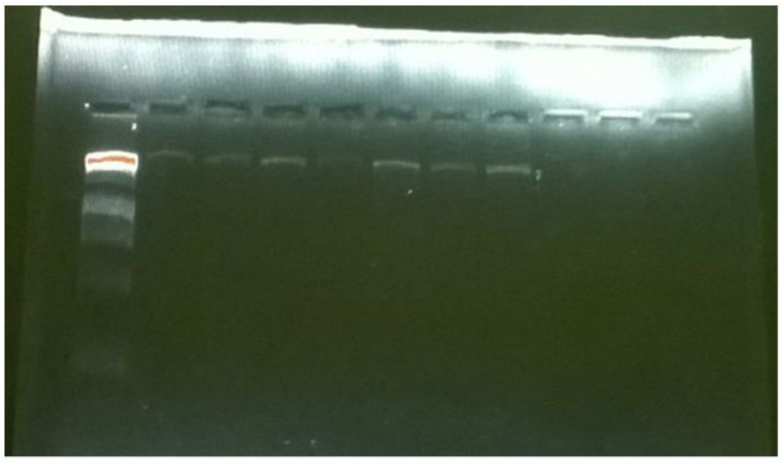


Concentrations resulted from Plasmid mini-prep (chloromphenicol resistance parts):
Manual protocol:

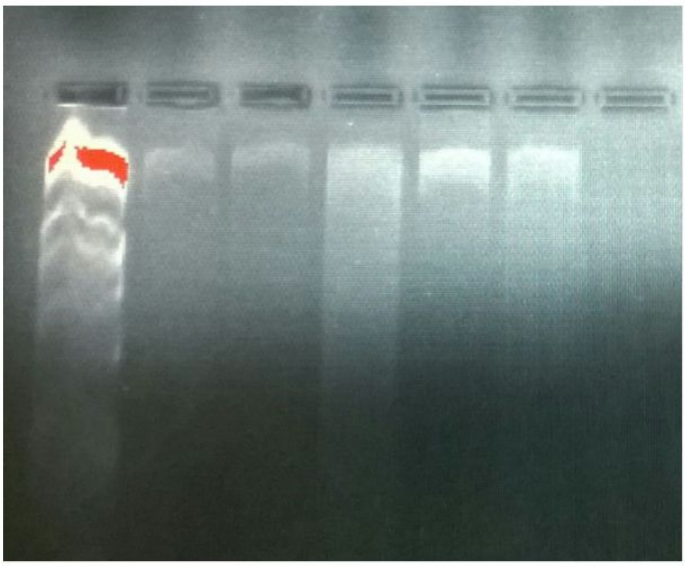
Part Number	Absorbance(260nm)	DNA concentration(ug/ul)
C1	0.184	0.92
C2	0.123	0.615
C3	0.17	0.85
C4	0.162	0.81
C5	0.18	0.9
C6	0.067	0.335
C7	0.091	0.455
C8	0.128	0.64
C9	0.082	0.41
C10	0.167	0.835
C11	0.109	0.545
C12	0.113	0.565

Using Kit

Part Number	Absorbance(260nm)	DNA concentration(ug/ul)
1	0.022	0.11
2	0.014	0.07
3	0.004	0.02
4	0.011	0.055
5	0.006	0.03
6	0.006	0.03
7	0.012	0.06
8	0.013	0.065
9	0.013	0.065
10	0.058	0.29
11	0.021	0.105
12	0.029	0.145



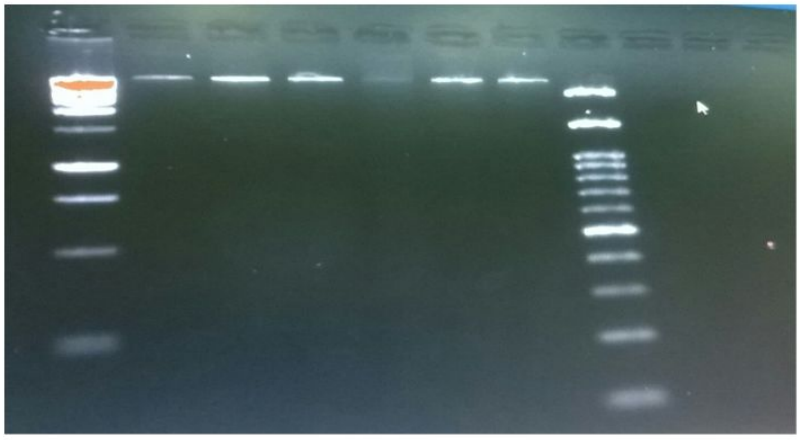
Visualizing mini-preped plasmids (for C resistance parts)
1)1kb ladder 2) to 8) plasmids with parts from C1 to C7
Note : the marker separation isn't good !



Visualizing mini-preped plasmids (for C resistance parts)
1)1kb ladder 2) to 9) plasmids with parts from C8 to C12

Concentrations resulted from Plasmid mini-prep (Ampicillin resistance parts):

Part Number	Absorbance(260nm)	DNA concentration(ug/ul)
1	0.026	0.13
2	0.016	0.08
3	0.026	0.13
4	0.063	0.315
5	0.034	0.17
6	0.015	0.075



Visualizing mini-preped plasmids (for A resistance parts)
1)1kb ladder. 2) to 7) plasmids with parts from A1 to A6. 8)100 bp ladder
Note : the 100bp ladder is separated probably not misleading as the 1kb ladder!

Assembly trials :

<u>Trial No. 1 (inter-lab):</u>

-Preparation of Enzymes Master mix for plasmid backbone:
25 ul total volume (for 5 Rxs)
(5 ul Buffer H
0.5 ul pstI
0.5 ul ECORI

19 ul dH2O)
-to digest plasmid : (4 ul linearized plasmid &4 ul enzyme master mix)

We are supposed to cut part A (with ECORI & SpeI) & part B (with XbaI & PstI)
Sadly we couldn't obtain the 4 enzymes from one company, So we had to do the double digestion in separate steps
ECORI & PstI (from Takara), SpeI & XbaI (fast digest from Thermo scientific)

*Digestion of part A (parts C8, C9, C10 the promoters for inter-lab)
<u>step 1 :</u>
- Reaction components :
SpeI Rx. 10 ul total Rx:
1ul Fast digest buffer (10x)
0.5 ul SpeI
DNA (vol. that equals 500 ng DNA)
adH2O complete vol. till 10 ul
- 37 C for 5 minutes then heat inactivation 65 C for 20 minutes
<u>Step 2</u> :
- Reaction components :
ECORI Rx (10ul total vol. added to the previous 10 ul)
0.5 ul enzyme
4 ul H buffer
5.5 ul dH2O
- incubation for 37 C for 1 hr

*Digestion of part B (part A the GFP generator for inter-lab)
<u>step 1 :</u>
- Reaction components :
XbaI Rx. 10 ul total Rx:
1ul Fast digest buffer (10x)
0.5 ul XbaI
DNA (vol. that equals 500 ng DNA)
dH2O complete vol. till 10 ul
- 37 C for 5 minutes then heat inactivation 65 C for 20 minutes
<u>Step 2 :</u>
- Reaction components :
PstI Rx (10ul total vol. added to the previous 10 ul)
0.5 ul enzyme

4 ul H buffer
5.5 ul dH2O
- incubation for 37 C for 1 hr

Ligation Rx:
Using Rapid ligation kit "thermo scientific "

Transformation results : negative

Trial No. 2 :
in the second trial we have left parts A & B cut by ECORI & PSTI respectively Over night
then we have carried out the Fast digest Reaction , then ligated the parts with a pSB1T3 vector

transformation results : negative

Trial No. 3:
in the third trial we have searched for a buffer that can solve us the different restriction enzyme requirements issue :
we have used Multi-Core buffer but no results obtained

Trial No. 4 :
Using Tango Buffer as following :
ECOR1 & SpeI double digestion Rx. :
1) first digest in 10x Tango buffer with SpeI
2) Incubate in 37 C for 1 hr
3) then add 10x Tango buffer to a final 2x conc. & EcoRI (V= A/8 , A is the vol of Rx. Mixture)

PstI & XbaI double digestion Rx.:
1)first Rx. Total Vol. 10 ul :
1ul Tango buffer 10x
0.5 ul enzyme SpeI
up to 1 ug DNA
nuclease free water (up to 10 ul)
2) then add 1.3 ul Tango buffer 10x
1.5 ul ECORI
& complete till 20 ul with nuclease free water

ligation using the Rapid ligation kit
transformation
Results : negative


PCR "amplifying the Parts" :

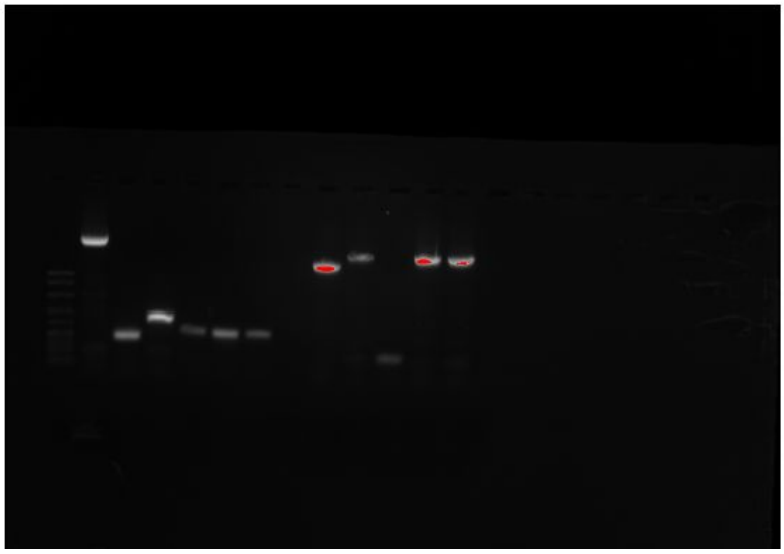
Total vol. of Rx. : 25 ul
Emerald PCR master mix 2x : 12.5 ul
Fwd primer (Conc. 100nM) : 1 ul
Rev primer (Conc. 100nM) :1 ul
DNA Conc. : vol. equivalent to 100 ng
dH2o : complete the vol. till 25 ul

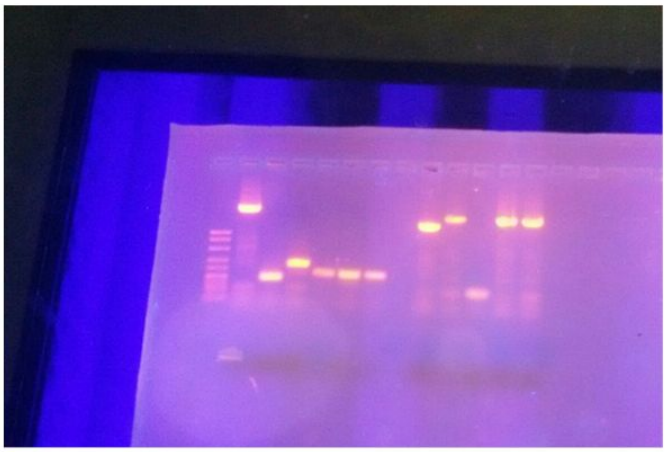
PCR Program:

Step	Time	Degree " C"
Initial denaturation	3 min	94
Denaturation	45 sec	94
Annealing	35 sec	59
Extension	70 sec	72
Final extension	5 min	72

Results :

Image Report: Dell 2015-09-12 20hr 42min12-sep pcr up parts





Gel map: 1) 100bp ladder 2)C1, 3)C2, 4)C5, 5)C8, 6)C9, 7)C10, 8)space, 9)A1, 10)A2, 11)A3, 12)A5, 13)A6

Concentration resulted from gel purification protocol :

Part Number	Absorbance (260nm)	DNA concentration (ug/ul)
A1	0.035	0.175
A2	0.029	0.145
A3	0.46	2.3
A5	0.064	0.33
A6	0.031	0.155
C1	0.037	0.185
C2	0.024	0.12
C5	0.025	0.125
C8	0.031	0.155
C9	0.048	0.24
C10	0.049	0.245

On 13th Sep. finally we managed to obtain ECORI & PstI as a fast digest "from thermo scientific"

Restriction digestion of plasmid backbone (by E & P)
restriction digestion of part C1 (by S & E)
restriction digestion of part A1 (by P& X)

ligation of Parts & transformation : Finally positive ones !!!!

Culturing the parts, mini prep
then repeating the cutting using the Fast digest enzymes
& adding (A1+ C1) to IL-10 one time & to the GFP generator the other time

Transformation results :