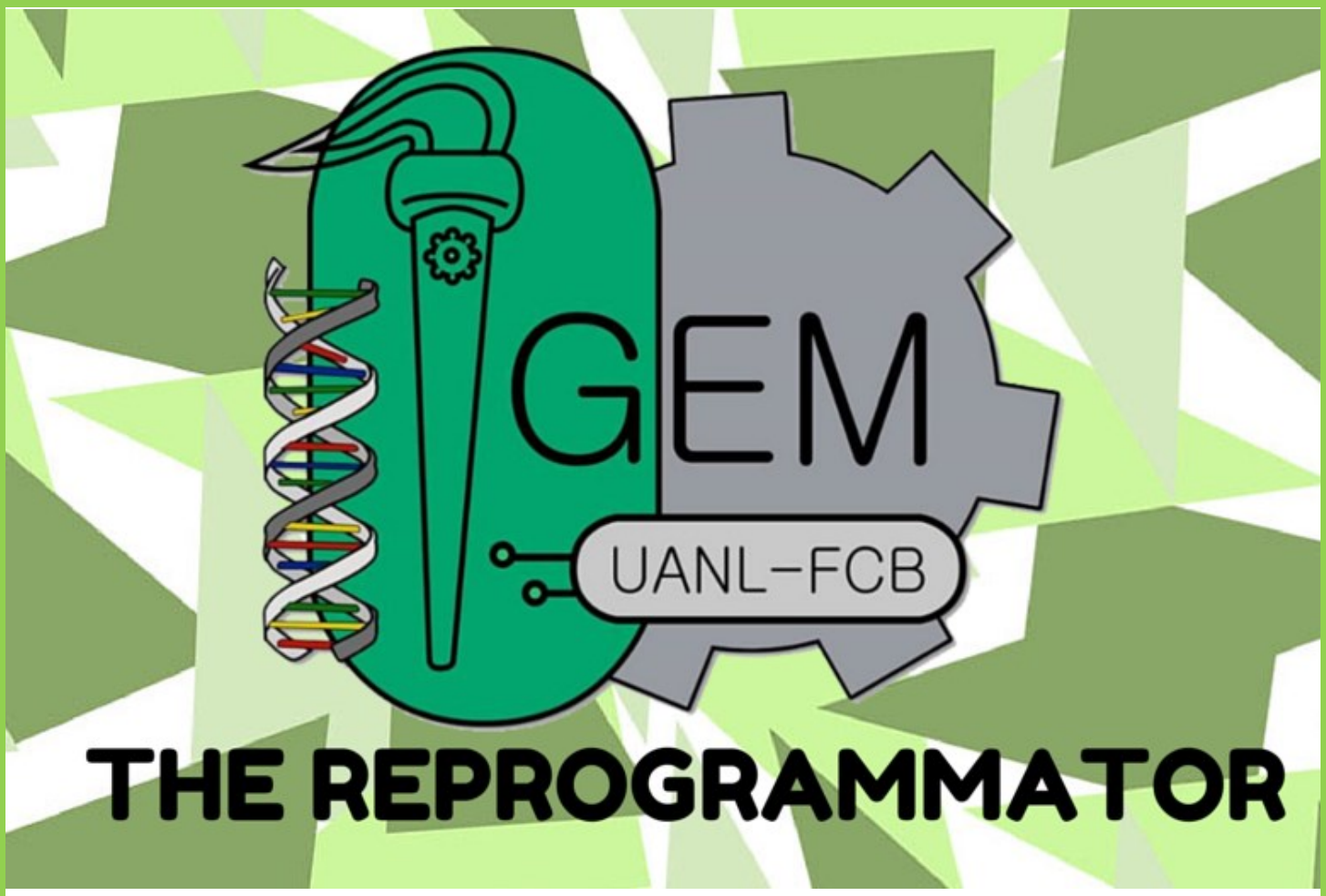


NOTEBOOK

JUNE 2014

25 WEDNESDAY

From the distribution kit 2014, we selected the following DNA for interlab work:

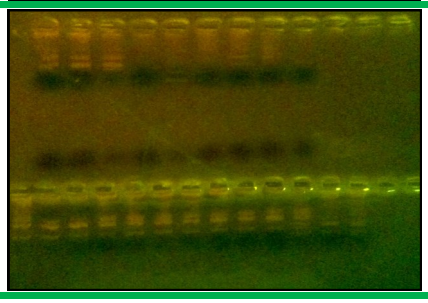
BBa_K82300S
BBa_K823012
BBa_120260
BBa_E0240

Those DNAs were used to transform *E. coli* TOP10 competent.

26 THURSDAY

We selected colonies from plates and cultured them in LB media.

27 FRIDAY



We made Minipreps of cultures to work with interlab. Then, those DNAs were stored at 4°C.

Gel #790

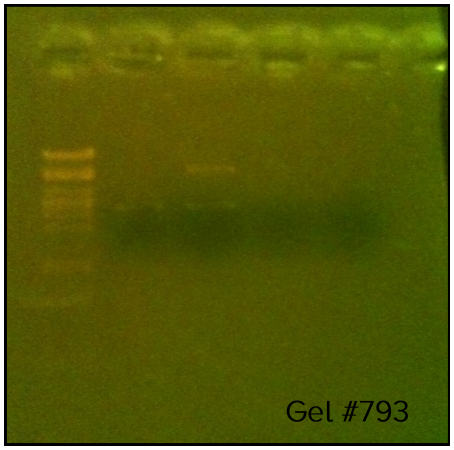
30 MONDAY

We selected DNAs based in **Gel #790** and cut them as is shown:

K823005	
Reactives	µL
DNA	4
SpeI	0.3
PstI	0.3
Buffer O	2
H_2O	13.6

K823012	
Reactives	x 2 (µL)
DNA	4
XbaI	0.3
PstI	0.3
Buffer O	2
H_2O	13.6

E0240	
Reactives	x 2 (µL)
DNA	4
XbaI	0.3
PstI	0.3
Buffer M	2
H_2O	13.6



We considered making the next abbreviation: **K823005**, the strong promoter, is 20K; **K823012**, the weak promoter, is now 22I; E0240, the CDS for GFP, is 24B.

We must to do new cultures and minipreps of 24B.

JULY 2014

2 WEDNESDAY

Minipreps from tubes 24B (BBa_E0240)
We considered that it is needed to obtain new cultures of 24B.

3 THURSDAY

Minipreps from tubes 24-B. This time new persons were included (Enrique and Claudia) in the protocol to see a change.

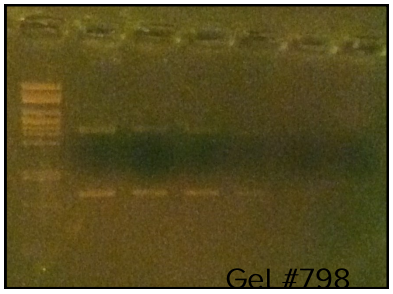
Also, we wanted to do an assay. We prepared tubes with *E. coli* DH5α and GM1061. We used LB and LB + Glycerol.

4 FRIDAY

Minipreps from tubes with bacteria DH5α and 1061.

7 MONDAY

Reactives	x 5 (µL)
DNA	4.0
XbaI	0.3
PstI	0.3
Buffer M	2.0
H_2O	13.6



Digestion, quantification, and ligation of the piece 24B for interlab.

Colonies were taken of DH5-α and 1061 and culture them in LB and LB + Glycerol. Also, this day we made ligation of 20K-24B and 22I-24B.

8 TUESDAY

- Miniprep and quantification of cultures of DH5-α and 1061,
- Measurement of OD_{600} and DNA concentration.
- Transformation of BBa_120260 (clones called 18A).

9 WEDNESDAY

- Miniprep and quantification of DH5-α and 1061.
- Growth of 10 colonies of 4-18A, in order to measure GFP act

10 THURSDAY

- GFP measurements for the piece 4-18A for Interlab.
- We pick cultures of 22I-24B, 20K-24B and 18-A to measure the next day.

11 FRIDAY

- GFP measurements of 22I-24B, 20K-24B and 18-A.
- We did not obtain fluorescence in 20K-24B, so we pick new colonies andwere culture in LB medium.
- Minipreps from the cultures:

4 cultures were fluorescent and the miniprep (22I-GFP) results.

14 MONDAY

Reactive	x 1 (µL)
20K	5.0
24B	2.4
Buffer	2.0
Ligase	1.0
H_2O	9.6

No growth of cultures 20K-24B.
Transformation of the ligation from ligation of 20K and 24B.

15 TUESDAY

Minipreps from cultures 20K-24B, but no results were obtained.
We made miniprep and quantification to the last set of Top10 and GM1061 of bacteria.

JULY

2014

17 THURSDAY	Cultures of 4-18A (GFP generator).
21 TUESDAY	Transformation of ligation 20K-24B. Cultures of 20K and 24B were prepared in case transformation fails. A new ligation of 20K-24B was made, just in case (2).
22 WEDNESDAY	The colonies of 20K-24B that growth were picked. Minipreps from cultures 20K and 24B. Digestions for the ligations (3).
23 THURSDAY	GFP measurement (20K-24B) of probable clones, although we did not observe fluorescence.
31 THURSDAY	We tried for the last time a digestion of 24B and 20K DNA. Those digestions were incubated all night.

AUGUST 2014

<table>
<tr><td>1
FRIDAY</td><td>We made the ligation of promoter 20K and CDS 24B.
<table><tr><th>Reactive</th><th>x 1 (µL)</th></tr><tr><td>24B</td><td>4.9</td></tr><tr><td>20K</td><td>5.0</td></tr><tr><td>Ligase</td><td>1.0</td></tr><tr><td>Buffer</td><td>2.0</td></tr><tr><td>H_2O</td><td>7.1</td></tr></table></td></tr>
<tr><td>2
SATURDAY</td><td>No colonies growth, so we decided preparing new competent cells.</td></tr>
<tr><td>4
MONDAY</td><td>Transformation of ligation 20K-24B (8/1/14).</td></tr>
<tr><td>14
THURSDAY</td><td>We have a delay due to issues and have not asked for the synthetic TALEN.
Also, we are having problems to obtain the GFP generator with the strong promoter. So it is needed to solve this problem to work properly on interlab</td></tr>
<tr><td>28
THURSDAY</td><td>12 cultures, 11 from the KB clones and one from the control 18A, were prepared.</td></tr>
<tr><td>29
FRIDAY</td><td>The GFP fluorescence, from the previous day cultures, was measured on 96 plate wells.</td></tr>
</table>

SEPTEMBER 2014

10 WEDNESDAY	This day we did a trade with **TEC-Monterrey team,** so we obtained the clone that we needed and they received the clone that they required to interlab.
14 SUNDAY	**14 cultures were prepared:** • 4 from 18A clones • 4 from KB clones • 4 from IB clones • 1 from K • 1 from D2
15 MONDAY	The cultures from the previous day were analyzed on **96 plate** wells to measure GFP fluorescence.
17 WEDNESDAY	**21 cultures were prepared to be measured the next day:** • 6 of 18-A • 6 of 20K-24B • 9 of 20K-24B
18 THURSDAY	**18 cultures were prepared:** • 6 from AB clones • 6 from IB clones • 6 from KB clones
19 FRIDAY	The fluorescence of the 18 cultures, from the previous day, plus 3 KB colonies was measured on a 96 plate well. This was the final day to send results to **interlab,** so we gathered the last data and send it.

OCTOBER 2014

1 WEDNESDAY

Tec-Monterrey team asked us for help to construct several of their parts in pSB1C3.

7 SUNDAY

Ligations for Tec-Monterrey team.

Reactives	X 1 (µL)
C4	2.4
IC3	2.0
Ligase	1.0
Buffer T4	2.0
H_2O	12.6

20.0 µl

Reactives	X 1 (µL)
C6	1.6
IC3	2.0
Ligase	1.0
Buffer T4	2.0
H_2O	13.4

20.0 µL

Reactives	X 1 (µL)
C5	2.6
IC3	2.0
Ligase	1.0
Buffer T4	2.0
H_2O	12.4

20.0 µL

Reactives	X 1 (µL)
C7	1.7
IC3	2.0
Ligase	1.0
Buffer T4	2.0
H_2O	13.3

20.0 µL

Reactives	X 1 (µL)
C8	2.3
IC3	2.0
Ligase	1.0
Buffer T4	2.0
H_2O	12.7

20.0 µL

Reactives	X 1 (µL)
C9	3.0
IC3	1.4
Ligase	1.0
Buffer T4	2.0
H_2O	12.6

20.0 µL

8 MONDAY

We transformed pSB1C3 in TOP10.
We selected colonies from Tec-Monterrey plates.

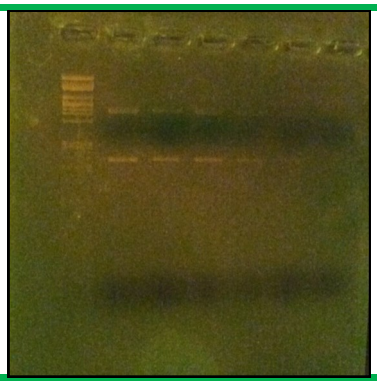
9 WEDNESDAY

We did miniprep to cultures of Tec-Monterrey and gave them. Also, we did miniprep to have our pSB1C3.

10 THURSDAY

Finally, the primers have arrived. We have been in hurry and did as fast as we could the PCRs.

11 FRIDAY



We did a electrophoresis to prove that PCR was successful.

13 WEDNESDAY

The PCR product was digested with DpnI and after 2 hours, we ligated the fragments.

15 WEDNESDAY

We transformed the ligations.
Also, we are preparing for some assays.


LB1
100 mL
1000
iGEM
GEM
UANL-FCB