Transformation of DH5 alpha with the "InterLab transformation protocol": DNA constructions were dried, so we resuspended them into 100µL nuclease-free water. We took 5µL of the product for 25µL of competent cells. All the experiment will be in ice to keep the competence of the cells.

Heat shock:
- 20” in ice
- 45s at 42°C
- 2” in ice

Recovery: 1mL of LB, 1h at 37°C. Preparation of LB Cam plates (30ug/mL).

Transformation of Top 10 (see the protocol of heat shock transformation above). We spread 100uL on LB cam plates.

Transformation seems have succeeded. We got few colonies on plates. So we seed on plates to get more. We should do a PCR colony to check our plasmids. We calculated the length of PCR products by two known primers: VR and VF2

Expected PCR products length(VR/VF2):
- Device 1, 2 and 3: 509 bp
- Negative control: 267 bp
- Positive Control: 1132 bp

Transformation of all samples from IGEM HQ. (a) Device 2 on the top, Negative control on the left, Positive control on the right; (b)Device 1 on the top, negative control on the left, Positive control on the right; (c) Device 3 on the top, Negative control on the left, Positive control on the right.
26/07/16:

*OD600 Reference point calibration
4 replicates of 100uL of Ludox (up)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0885</td>
<td>0.076</td>
<td>0.0757</td>
<td>0.0765</td>
</tr>
<tr>
<td>B</td>
<td>0.0635</td>
<td>0.0661</td>
<td>0.0651</td>
<td>0.067</td>
</tr>
</tbody>
</table>

- Mean value of Ludox Abs(600): 0.079175
- Mean value of H2O Abs(600): 0.065425
- Corrected Abs(600) = Ludox Abs(600) - H2O Abs(600): 0.01375
- Corrected Abs*Correction Factor = Reference Abs(600) = 0.01475
- Correction Factor: 1.0272727

4 replicates of -100uL of Ludox (down)

In OD600 mode:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SM1_1</td>
<td>SM1_3</td>
<td>SM1_5</td>
<td>SM1_7</td>
</tr>
<tr>
<td></td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>0.0785</td>
<td>0.0795</td>
<td>0.0767</td>
<td>0.0788</td>
</tr>
<tr>
<td>B</td>
<td>SM1_2</td>
<td>SM1_4</td>
<td>SM1_6</td>
<td>SM1_8</td>
</tr>
<tr>
<td></td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>0.069</td>
<td>0.0698</td>
<td>0.0648</td>
<td>0.0651</td>
</tr>
</tbody>
</table>

- Mean value of Ludox Abs(600): 0.078375
- Mean value of H2O Abs(600): 0.0671
- Corrected Abs(600) = Ludox Abs(600) - H2O Abs(600): 0.011275
- Corrected Abs*Correction Factor = Reference Abs(600) = 0.01475
- Correction Factor: 1.30820399

01/08/16:

Standard Curve in different relevant modes

Standard curve Optimal Gain 100%
The optimal gain value calculated is 44.

This will be the one that I keep!

But if we could redo the standard curve in different modes, I mean changing the orbitals (not the gain) in order to get a righter standard curve… It will be amazing but we’ll have to redo the cell measurement!
Pre-culture:
- 5mL LB
- 5uL Cam
- one colony from plate for T+, T-, devices 1, 2 and 3

13/08/16:
We measure the OD$_{600}$ of each pre-culture, the table bellow calculates the volumes of LB pre-culture to add to TB volume to get an final volume of 10mL with an OD$_{600}$=0.02

<table>
<thead>
<tr>
<th>sample</th>
<th>Abs$_{600}$ volume of preloading media</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive control</td>
<td>1.50</td>
</tr>
<tr>
<td>negative control</td>
<td>0.49</td>
</tr>
<tr>
<td>device 1</td>
<td>1.48</td>
</tr>
<tr>
<td>device 2</td>
<td>1.05</td>
</tr>
<tr>
<td>device 3</td>
<td>1.47</td>
</tr>
<tr>
<td>media+chl</td>
<td>0.004</td>
</tr>
<tr>
<td>media+chl 1X</td>
<td>0.004</td>
</tr>
<tr>
<td>media+chl 0,5X</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Then incubated them in 37°C and 2200rpm, taking samples each hour during six hours. Samples will be in ice then put in duplicates in 96well plate following the schema bellow.

We did it for Two media concentrations: 0,5 and 1 X.
For 0.5:

Results for 0.5 X concentration aren’t good too spare
Neither for 1 X concentration

But we observed a greater fluorescence values in 1X but lower OD_{600} values meaning the bacteria seem to grow better on 0.5X concentration.
The Flu/OD600 ratio was highest in 1X.
We cannot compare the values difference of replicates because it the second values are missing for the 1X concentration.

We did not add the chemical compound needed in the TB media.
Preparation of Phosphate buffered saline 1X solution

- 8g NaCl for final concentration [137mM]
- 0.2 KCl [2.7mM]
- 1.44g Na$_2$HPO$_4$ [1mM]
- 0.24g KH$_2$PO$_4$ [1.8mM]

Add 800µL dH$_2$O and mix. Then, adjust pH=7.4 (7.2 if necessary) with HCl. When the pH is good, qs 1L. Sterilize by autoclaving.

Preparing the FITC stock solution

Spin down the FITC stock (FITC=194.7µg in the tube). Add 1mL of 1X PBS to have a stock solution of FITC at 500µM. Incubation at 42°C during four hours. After, I let the FITC in 42°C overnight because it is not dissolve totally.

Preparing the FITC 1X solution

In a new 1.5mL tube, put 500µL FITC 2X with 500µL PBS 1X. We obtained a FITC 1X solution (250µM).

Preparing the serial dilution of the FITC 1X solution:

We did the serial dilution according the protocol from the interlab.

We obtained these values.

Cell growth

→ Precultures of 2 colonies for each construction
Precultures of Device 1, 2, 3, negative control and the positive control in 5mL LB + 1µL Cam (30µg/mL), overnight (around 16 hours), 37°C, 220 rpm.

Measuring of OD$_{600}$

<table>
<thead>
<tr>
<th></th>
<th>Negative control</th>
<th>Positive control</th>
<th>Device 1</th>
<th>Device 2</th>
<th>Device 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replica N°1</td>
<td>0,5611</td>
<td>0,5711</td>
<td>0,5811</td>
<td>0,5811</td>
<td>0,6011</td>
</tr>
<tr>
<td>Replica N°2</td>
<td>0,5811</td>
<td>0,6211</td>
<td>0,6211</td>
<td>0,6811</td>
<td>0,5811</td>
</tr>
</tbody>
</table>

Volume to take off for a final OD$_{600} = 0.02$

<table>
<thead>
<tr>
<th></th>
<th>Negative control</th>
<th>Positive control</th>
<th>Device 1</th>
<th>Device 2</th>
<th>Device 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replica N°1 (µL)</td>
<td>356</td>
<td>350</td>
<td>344</td>
<td>344</td>
<td>333</td>
</tr>
<tr>
<td>Replica N°2 (µL)</td>
<td>344</td>
<td>322</td>
<td>322</td>
<td>294</td>
<td>344</td>
</tr>
</tbody>
</table>

We add this volume in 10 ml 0.5x TB medium + Chloramphenicol (30µg/mL) in 50mL tube for the normal condition.

For all the samples (conditions with and without betaine and sorbitol), we took 250uL of each sample for the time t=0, incubate the cultures at 37°C and 220 rpm. Each hour, we took 250uL until the time t=6. We kept the samples in ice until the last one. Then, we put 200uL in the 96-well plate and we analyzed the OD and the fluorescence with a plate reader.

Absorbance in function of the time (condition without betaine and sorbitol)
Fluorescence intensity in function of the time (condition without betaine and sorbitol).

**30/08/16**

*Precultures of 2 colonies for each construction*
Precultures of Device 1, 2, 3, negative control and the positive control in 5mL LB + 1µL Cam (30µg/mL), overnight (around 16 hours), 37°C, 220 rpm.

01/09/16

*Preparation of 1x TB “Ready to use”*
Add 30mL of potassium phosphate solution in 300mL 1x TB “Step 1”.

*Preparation of 0.5x TB “Ready to use”*
Add 60mL 1x TB with 60mL of steril water. Filtration with 0.22um.

*Preparation of 0.5x TB with chloramphenicol*
Add 72uL chloramphenicol in 120mL 0.5x TB.

- **OD600 measurement**

<table>
<thead>
<tr>
<th>Replica n°1</th>
<th>target sample</th>
<th>target volume (mL)</th>
<th>Abs600</th>
<th>re volume of preload</th>
<th>volume of preloading media</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive control</td>
<td>0.6368</td>
<td>0.352174579</td>
<td>9.647825</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative control</td>
<td>0.6337</td>
<td>0.354107949</td>
<td>9.645892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>device 1</td>
<td>0.6513</td>
<td>0.343406593</td>
<td>9.656593</td>
<td></td>
<td></td>
</tr>
<tr>
<td>device 2</td>
<td>0.6543</td>
<td>0.341646737</td>
<td>9.658353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>device 3</td>
<td>0.673</td>
<td>0.331071015</td>
<td>9.668925</td>
<td></td>
<td></td>
</tr>
<tr>
<td>media+chl</td>
<td>0.0689</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Replica N°2
For the condition with betaine and the sorbitol, we did the next mix for a final volume = 100mL: 91.67mL 0.5x TB (with phosphate); 18.33 mL 5X sorbitol (2.5M); 916.67µL 100x betaine (500mM); 66µL cam (50mg/mL). Each hour, we took 250uL until the time t=6. We kept the samples in ice until the last one. Then, we put 200uL in the 96-well plate and we analyse the OD and the fluorescence with a plate reader.

We obtained the next result:

<table>
<thead>
<tr>
<th>sample</th>
<th>Abs600</th>
<th>% volume of preloading media</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive control</td>
<td>0.6925</td>
<td>0,320718 9,679282</td>
</tr>
<tr>
<td>negative control</td>
<td>0.6491</td>
<td>0,344709 9,655291</td>
</tr>
<tr>
<td>device 1</td>
<td>0.6914</td>
<td>0,321285 9,678713</td>
</tr>
<tr>
<td>device 2</td>
<td>0.7542</td>
<td>0,291843 9,708157</td>
</tr>
<tr>
<td>device 3</td>
<td>0.6546</td>
<td>0,341472 9,658528</td>
</tr>
<tr>
<td>media+cHl</td>
<td>0.0639</td>
<td></td>
</tr>
</tbody>
</table>

Absorbance in function of the time (condition with betaine and sorbitol)

Fluorescence intensity in function of the time (condition with betaine and sorbitol).