BBa_K116001 - nhaA promoter

Characterization experiments on BBa_K116001 - UNIPV-Pavia Team (test performed by M. Meroso, G. Zambianchi)

Introduction

BBa_K116001 from iGEM 2008 NYMU-Taipei.
We received the BioBrick measurement system (which has a GFP protein generator downstream) of BBa_K116001 from iGEM HQ in September (it is called BBa_K116002). The bacterial strain that contained the plasmid was NEB 10-beta, so we decided to transform it into E. coli TOP10.
We wanted to perform some experiments to better understand how it works and if can be successfully used.
We performed several experiments with different LB medium and we got almost the same results. We used:

- LBK (NaCl 0M) (pH 5.5 - 6.6 - 7.5 - 8.5)
- LB NaCl 70mM (pH 5.5 - 6.6 - 7.5 - 8.5)
- LB NaCl 171mM (pH 5.5 - 6.6 - 7.5 - 8.5)
- LB NaCl 250mM (pH 5.5 - 6.6 - 7.5 - 8.5)
- LB NaCl 600mM (pH 10 - 11.2)

Experiment Na+ 0M

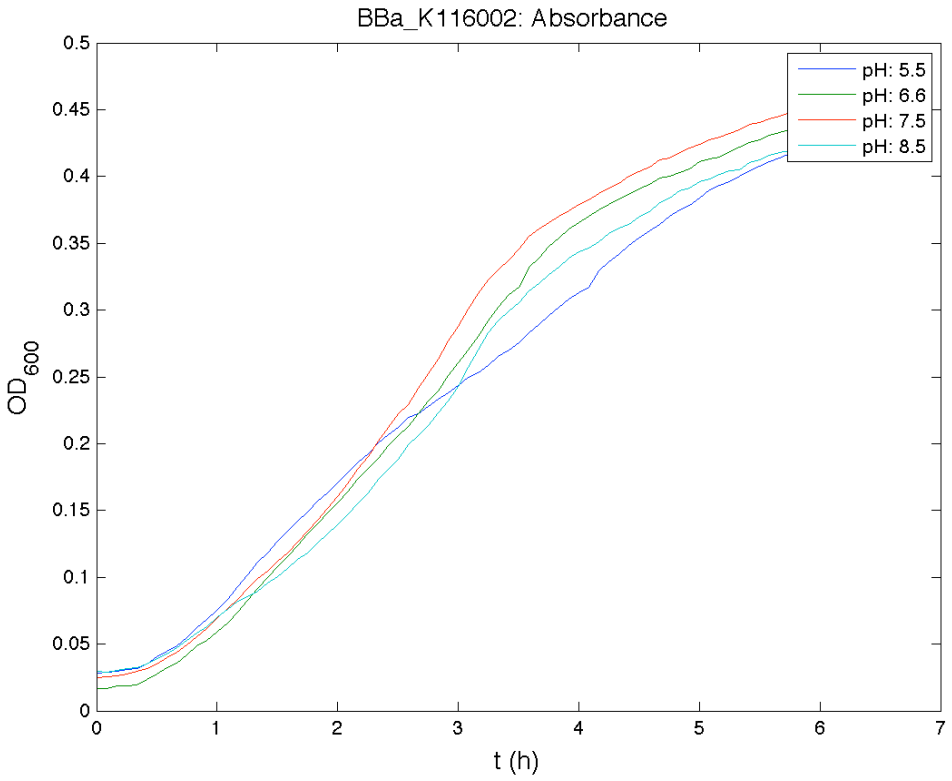
Motivation

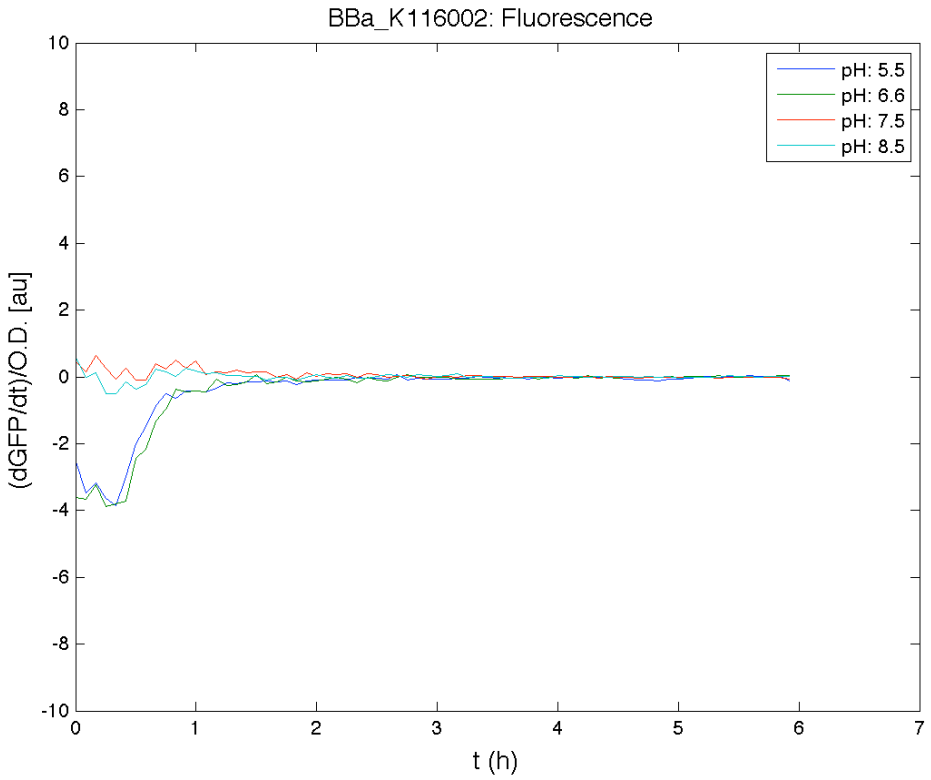
The working principle of the antiporter Na+/H+ channel described in [Rachel Karpel et al., Etana Padan et al., N. Dover et al.] makes the nhaA promoter a Na+ sensor and only under certain conditions (presence of Na+) a pH sensor.

Methods

- We prepared LBK (potassium - 87mM - instead of sodium) and adjusted pH using KOH and HCl to values 5.5, 6.6, 7.5 and 8.5.
- We inoculated 8ul of Invitrogen TOP10 containing BBa_K116002 into 4ml of LB + Amp and incubated overnight at 37°C, 220 rpm. We did the same for TOP10 with BBa_K173001 and BBa_B0033 inside.
- Next morning we put 50ul from each of the three falcon tubes into 5ml of LBK pH 6.6 and incubated again for about four hours and a half at 37°C, 220 rpm.
- We measured the final O.D. with TECAN Infinite F200 and diluted each genetic circuit into four falcon tubes with LBK at different pH (5.5 - 6.6 - 7.5 - 8.5) in order to obtain a same O.D. equal to 0,02 (12 falcon tubes overall).
- Then we performed a 6 hours' experiment with measurements of absorbance and fluorescence every 5 minutes with TECAN Infinite F200. Each value shown is the mean of three measurements and cultures were shaked for 15 seconds every five minutes.

Results




BBa_K116002: Fluorescence
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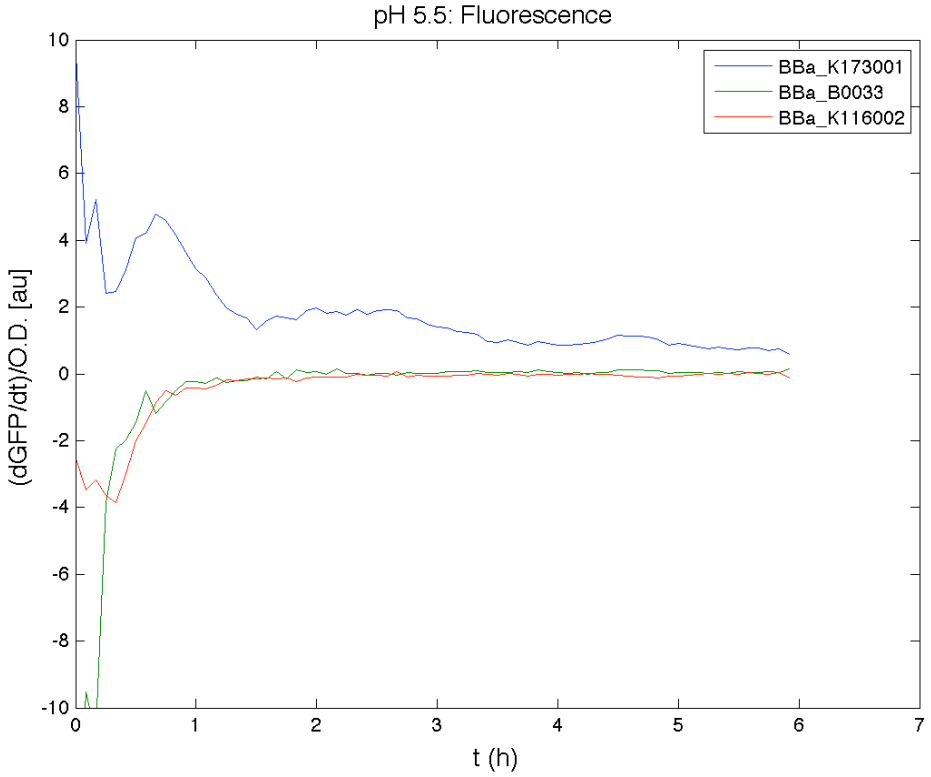
pH: 6.6
pH: 7.5
pH: 8.5
(dGFP/dt)/O.D. [au]
t (h)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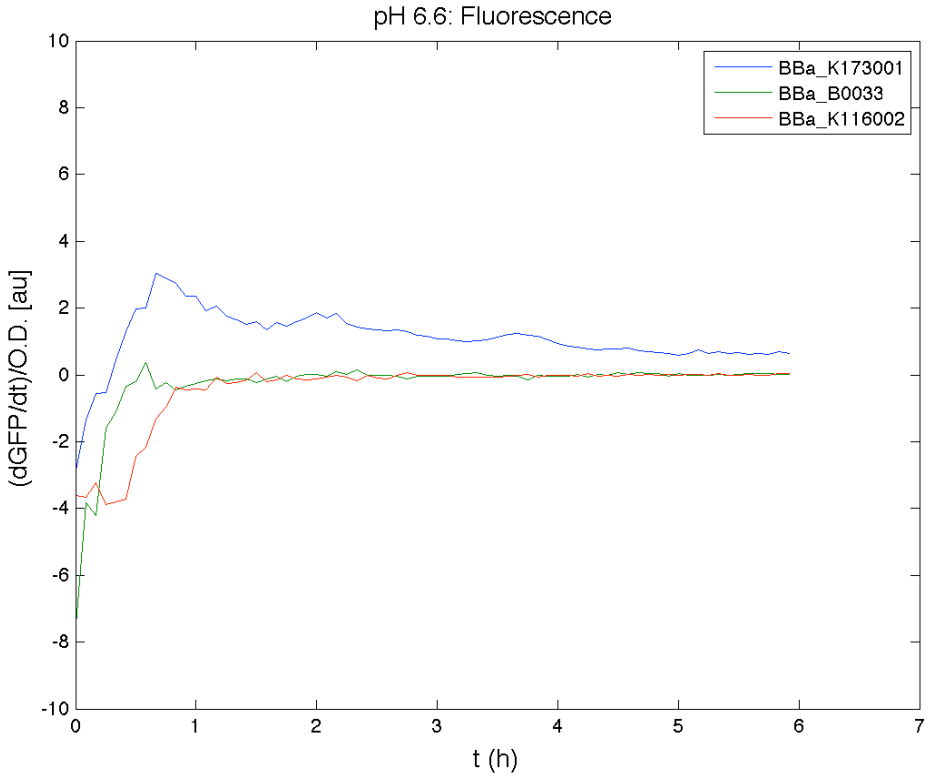
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)


pH 6.6: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)
10
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-2
-4
-6
-8
-10
0
1
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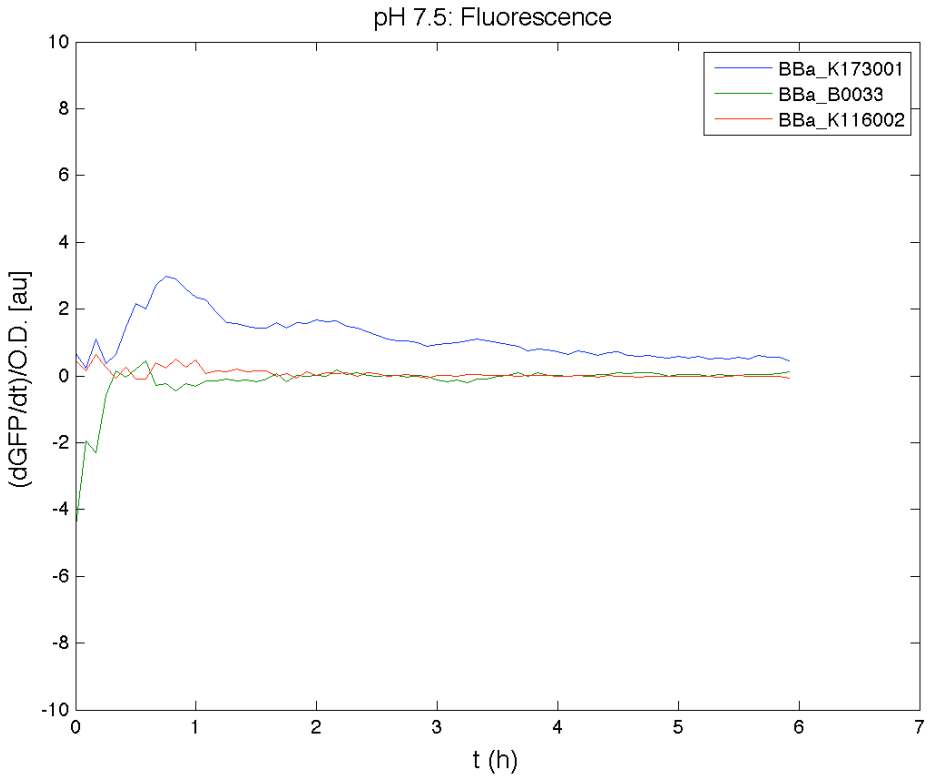
pH 7.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)
10
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-10
0
1
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3
4
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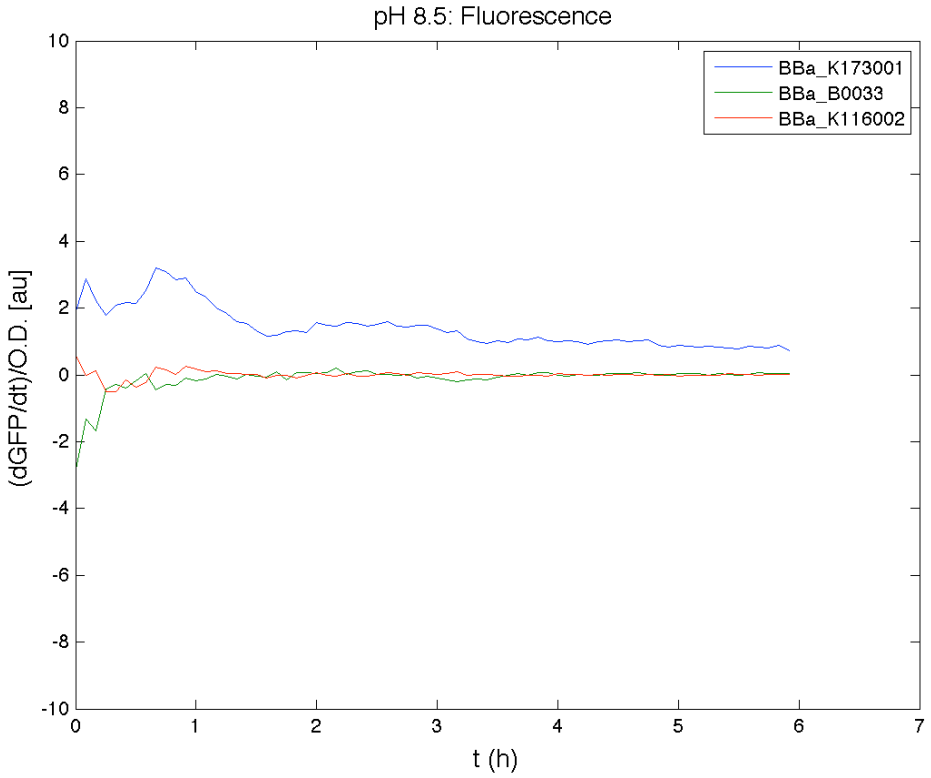
6
7



Comments

As you can see from graphs BBa_K116002 didn't produce any GFP. After a short transient in which we have some noise you can see that the GFP production rate goes to zero as well as the negative control (BBa_B0033). Positive control (BBa_K173001) has significantly higher production rate. So we can consider it a Na+ sensor and only secondarily a pH sensor.

Experiment Na+ 70mM

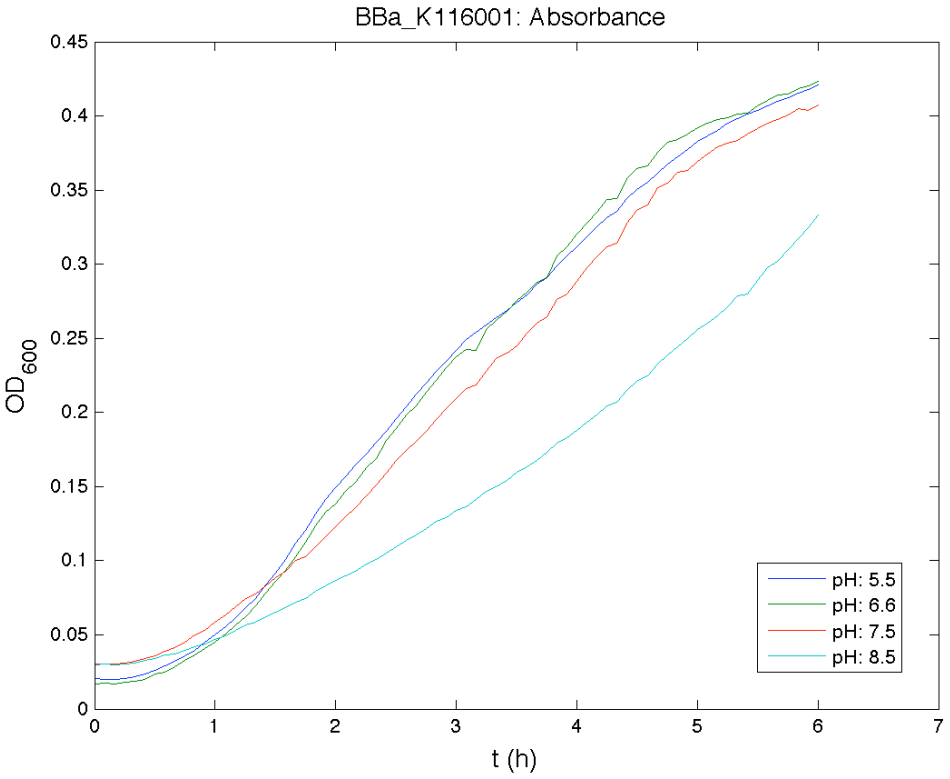
Motivation

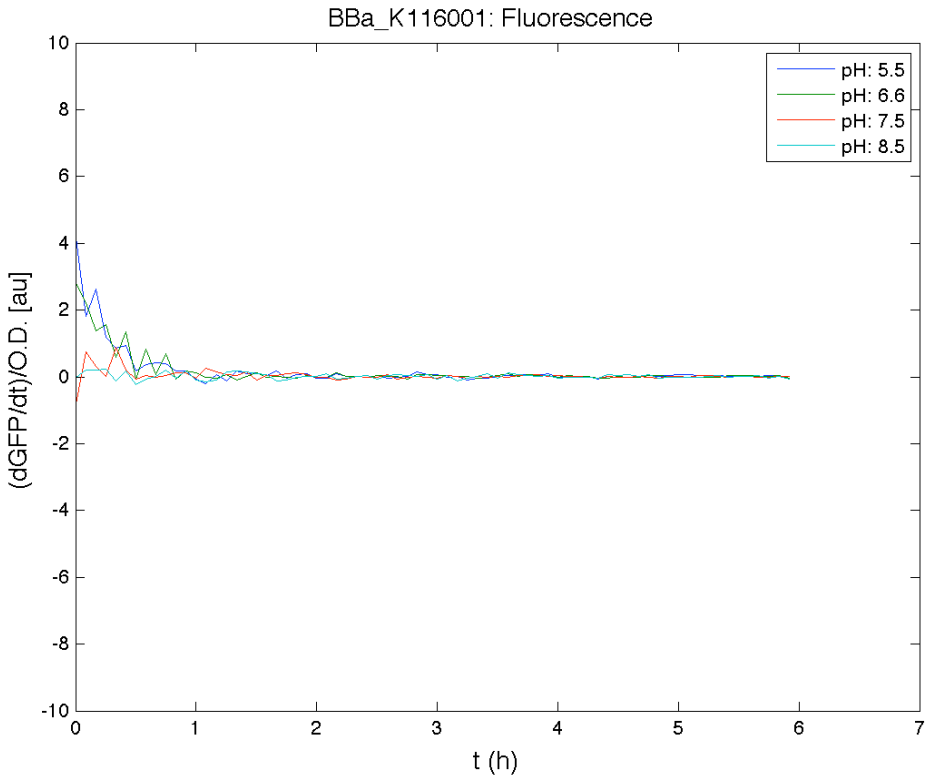
We'll try again to make E. coli producing GFP at the variation of pH in presence of Na+ 70mM.

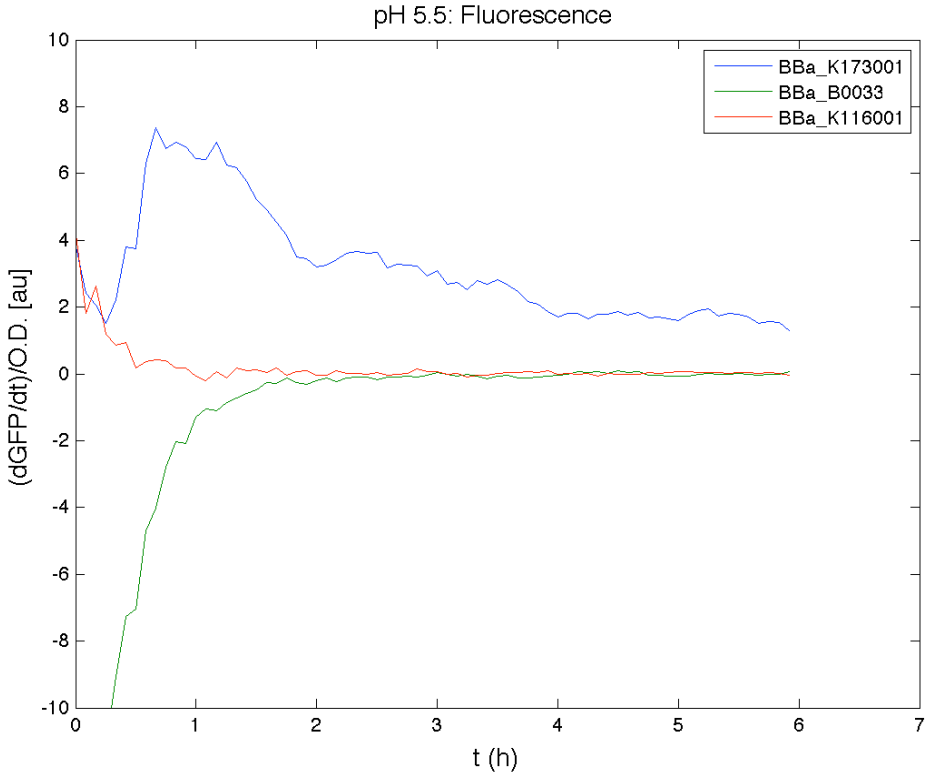
Methods

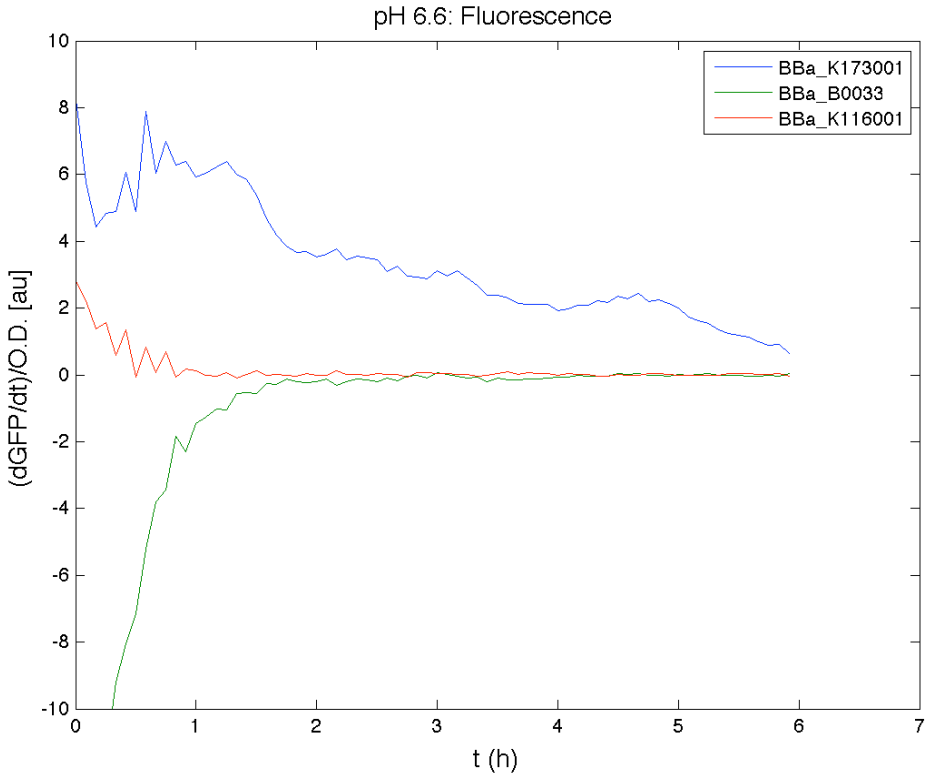
- We prepared LB NaCl 70mM and adjusted pH using KOH and HCl to values 5.5, 6.6, 7.5 and 8.5.
- We inoculated 8ul of Invitrogen TOP10 containing BBa_K116002 into 4ml of LB + Amp and incubated overnight at 37°C, 220 rpm. We did the same for TOP10 with BBa_K173001 and BBa_B0033 inside.
- Next morning we put 50ul from each of the three falcon tubes into 5ml of LB NaCl 70 mM pH 6.6 and incubated again for four hours and a half at 37°C, 220 rpm. After that we measured the final O.D. with TECAN Infinite F200 and diluted each genetic circuit into four falcon tubes with LB NaCl 70 mM at different pH (5.5 - 6.6 - 7.5 - 8.5) in order to obtain a same O.D. equal to 0,02 (12 falcon tubes overall).
- Then we performed an experiment of 6 hours' duration with measurements of absorbance and fluorescence every 5 minutes with TECAN Infinite F200. Each value shown is the mean of three measurements and cultures were shaked for 15 seconds every five minutes.

Results




BBa_K116001: Fluorescence
pH: 5.5
pH: 6.6
pH: 7.5
pH: 8.5
(dGFP/dt)/O.D. [au]
t (h)


pH 5.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116001
(dGFP/dt)/O.D. [au]
t (h)


pH 6.6: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116001
(dGFP/dt)/O.D. [au]
t (h)
10
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-2
-4
-6
-8
-10
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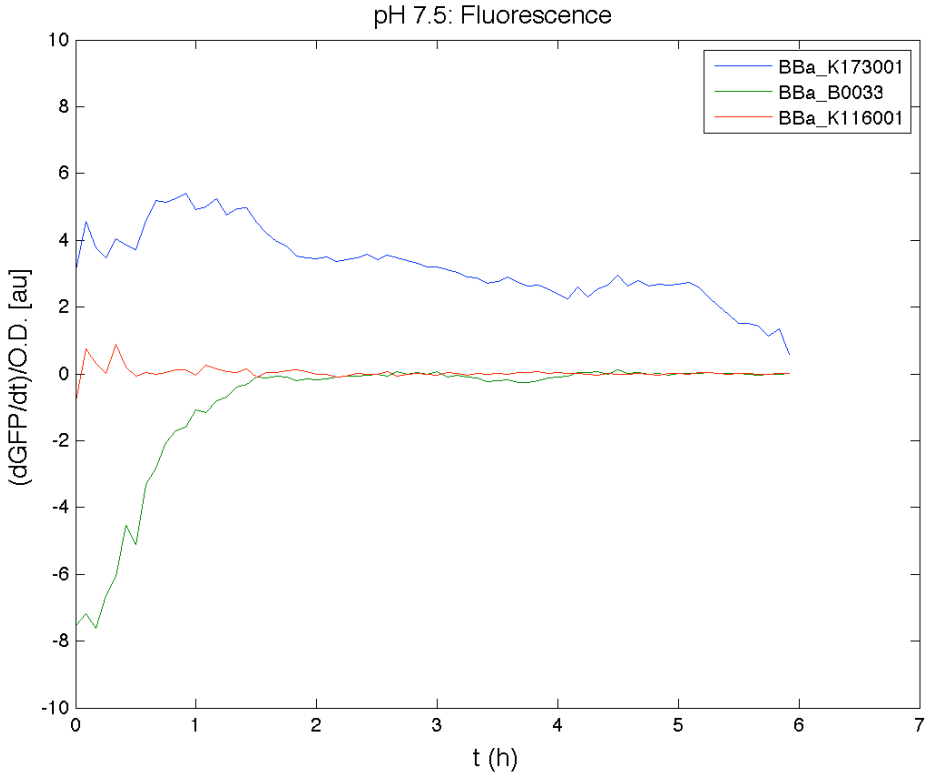
pH 7.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116001
(dGFP/dt)/O.D. [au]
t (h)
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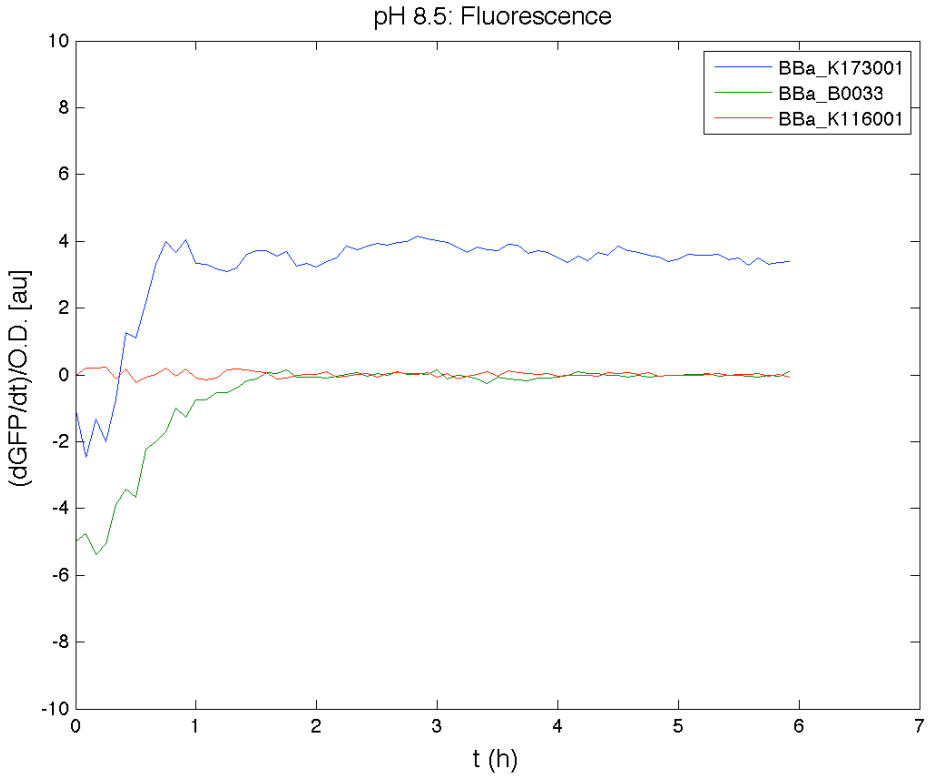


Comments

We didn't expect this. Again BBa_K116002 didn't produce any GFP as well as negative control (BBa_B0033): after the transient due to noise their GFP production rate goes to zero while positive control (BBa_K173001) has a significantly higher one. From literature ([Rachel Karpel et al., Etana Padan et al., N. Dover et al.]) this promoter should already activate at these Na+ concentrations.

Experiment Na+ 171mM

Motivation

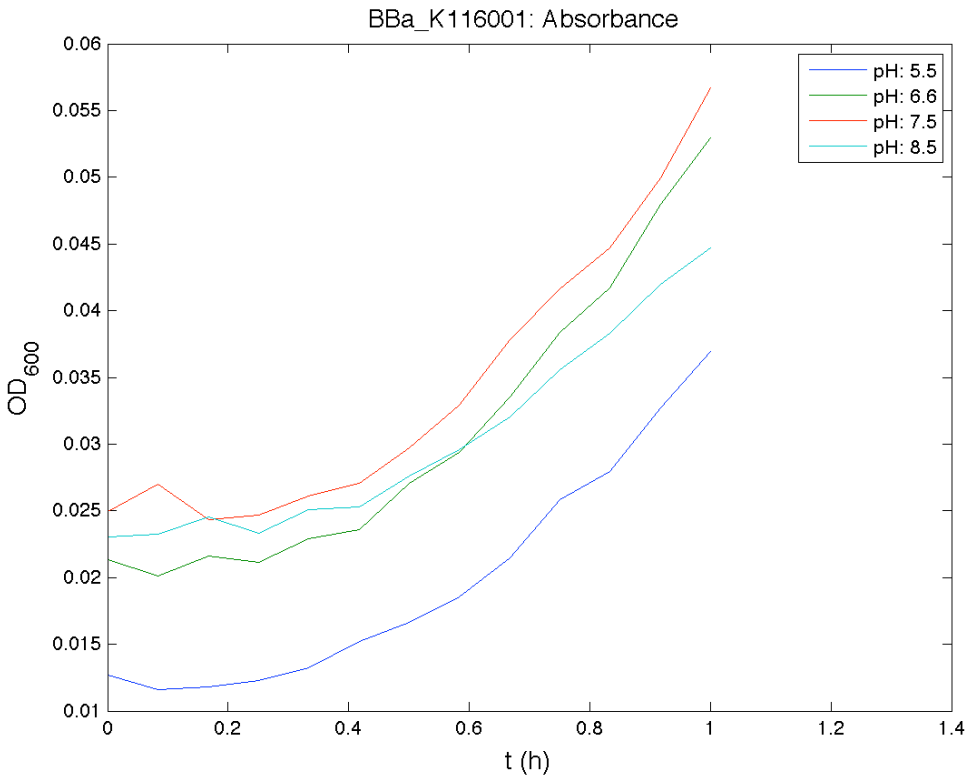
We'll try again to make E. coli producing GFP at the variation of pH in presence of Na+ 171mM.

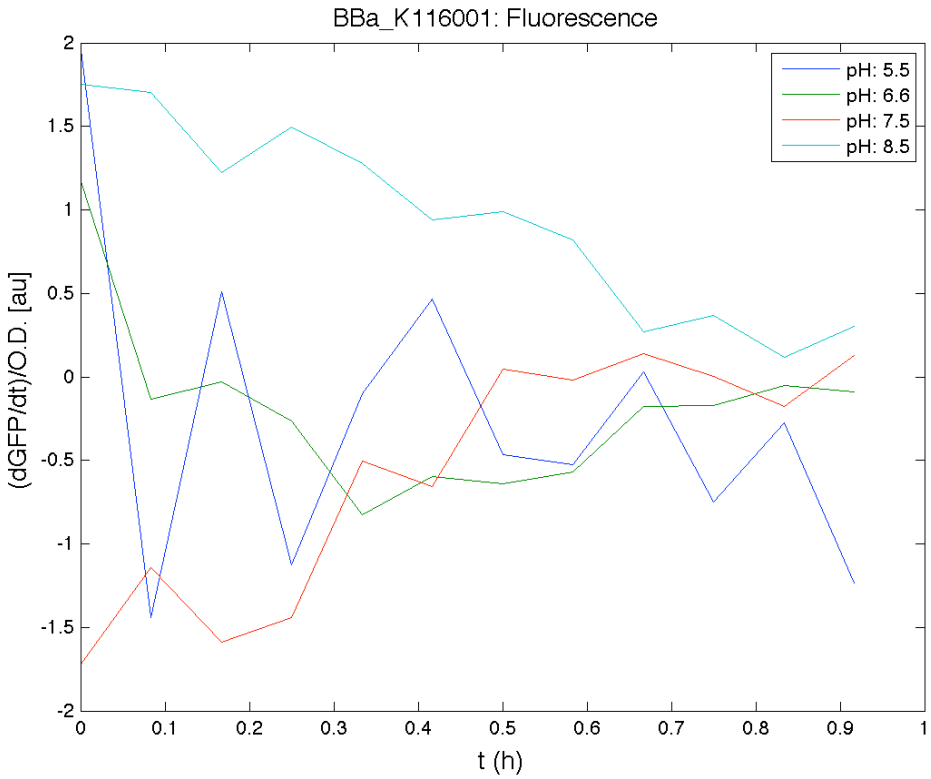
Methods

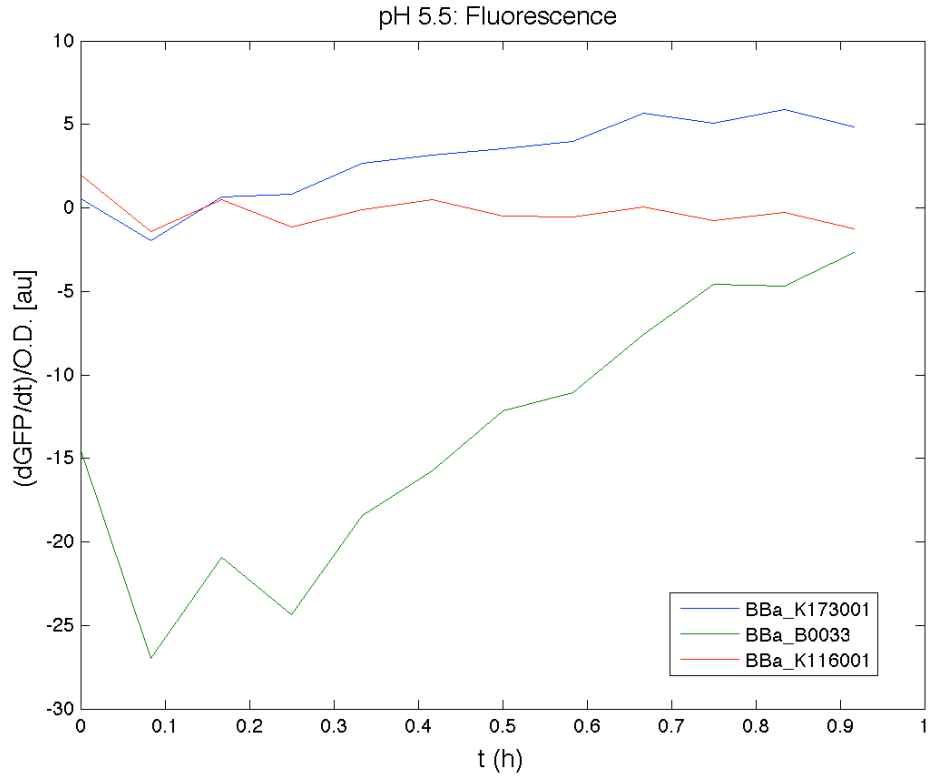
- We prepared LB NaCl 171mM and adjusted pH using KOH and HCl to values 5.5, 6.6, 7.5 and 8.5.
- We inoculated 8ul of Invitrogen TOP10 containing BBa_K116002 into 4ml of LB + Amp and incubated overnight at 37°C, 220 rpm. We did the same for TOP10 with BBa_K173001 and BBa_B0033 inside.
- Next morning we put 50ul from each of the three falcon into 5ml of LB NaCl 171 mM pH 6.6 and incubated again for four hours and a half at 37°C, 220 rpm. After that we measured the final O.D. with TECAN Infinite F200 and diluted each genetic circuit into four falcon tubes with LB NaCl 171mM at different pH (5.5 - 6.6 - 7.5 - 8.5) in order to obtain a same O.D. equal to 0,02 (12 falcon tubes overall).
- Then we performed an experiment of 6 hours' duration with measurements of absorbance and fluorescence every 5 minutes with TECAN Infinite F200. Each value shown is the mean of three measurements and cultures were shaked for 15 seconds every five minutes.

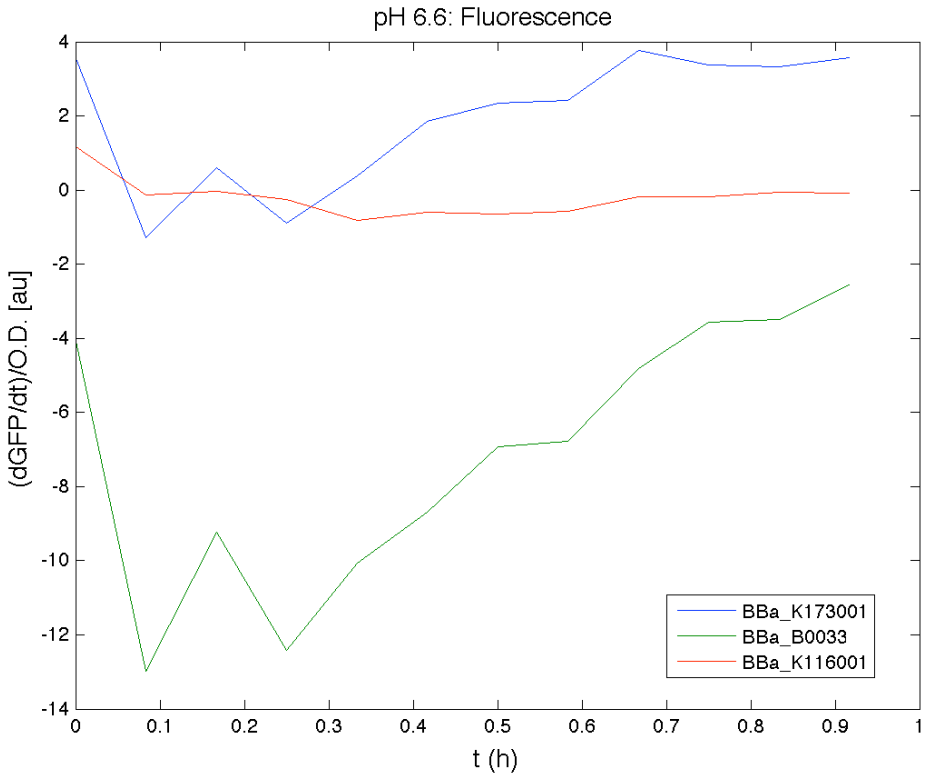
Results

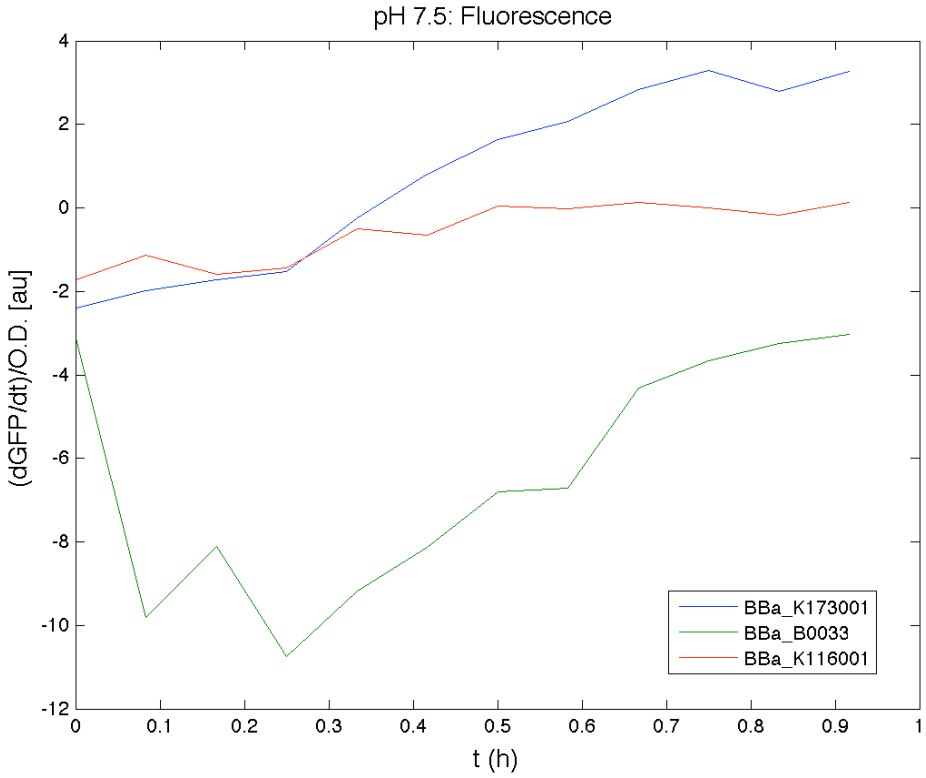
For some reason the PC powered off during the night so we lost almost all data. Here what we could recover.

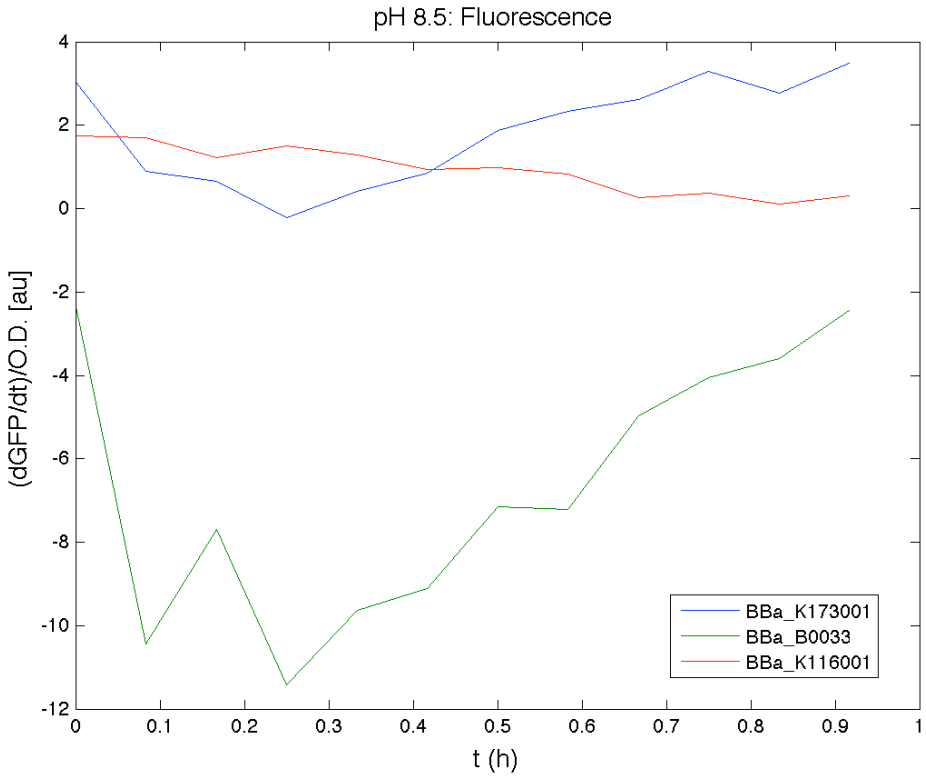



BBa_K116001: Fluorescence
pH: 5.5
pH: 6.6
pH: 7.5
pH: 8.5
(dGFP/dt)/O.D. [au]
t (h)

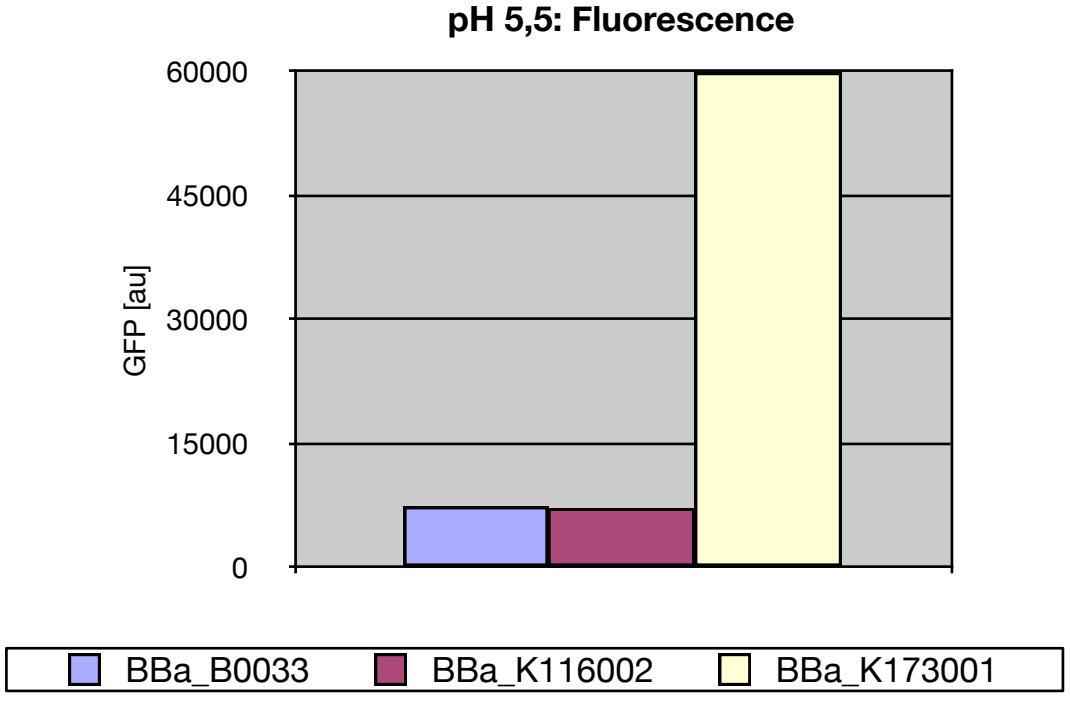

pH 5.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116001
(dGFP/dt)/O.D. [au]
t (h)

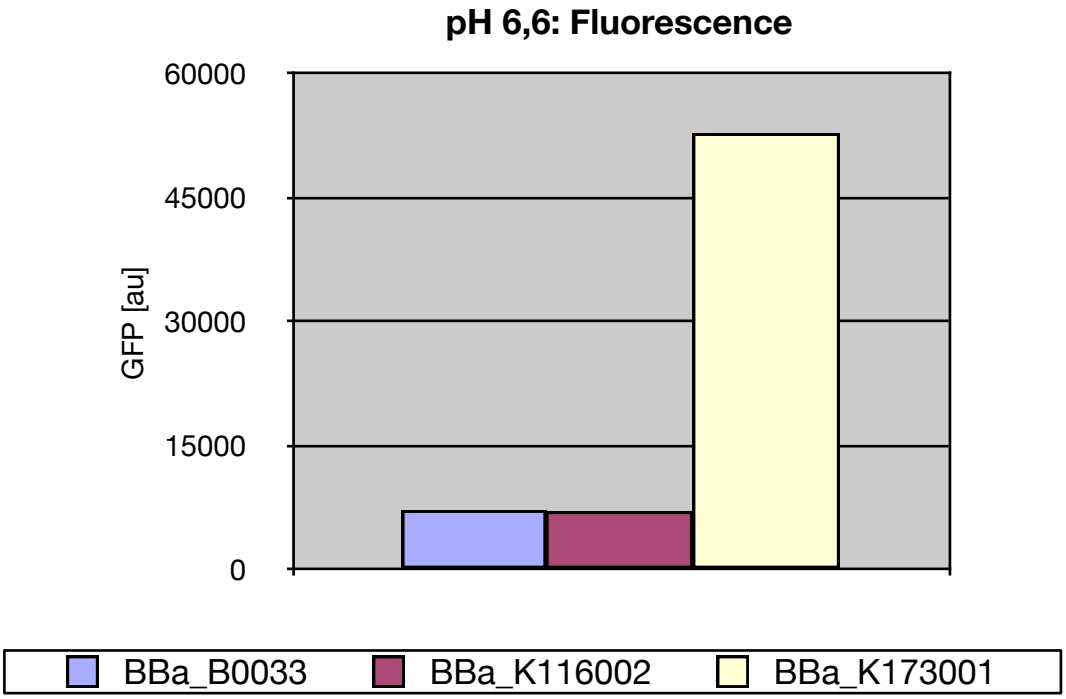

pH 6.6: Fluorescence
(dGFP/dt)/O.D. [au]
t (h)
BBa_K173001
BBa_B0033
BBa_K116001

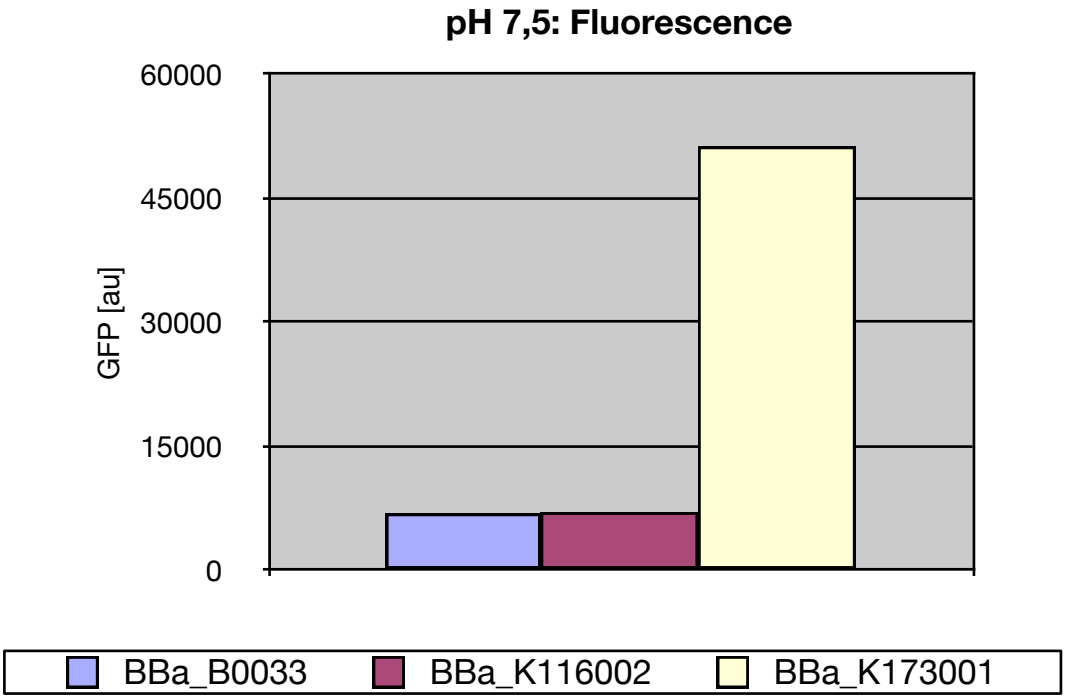

pH 7.5: Fluorescence
(dGFP/dt)/O.D. [au]
t (h)
BBa_K173001
BBa_B0033
BBa_K116001

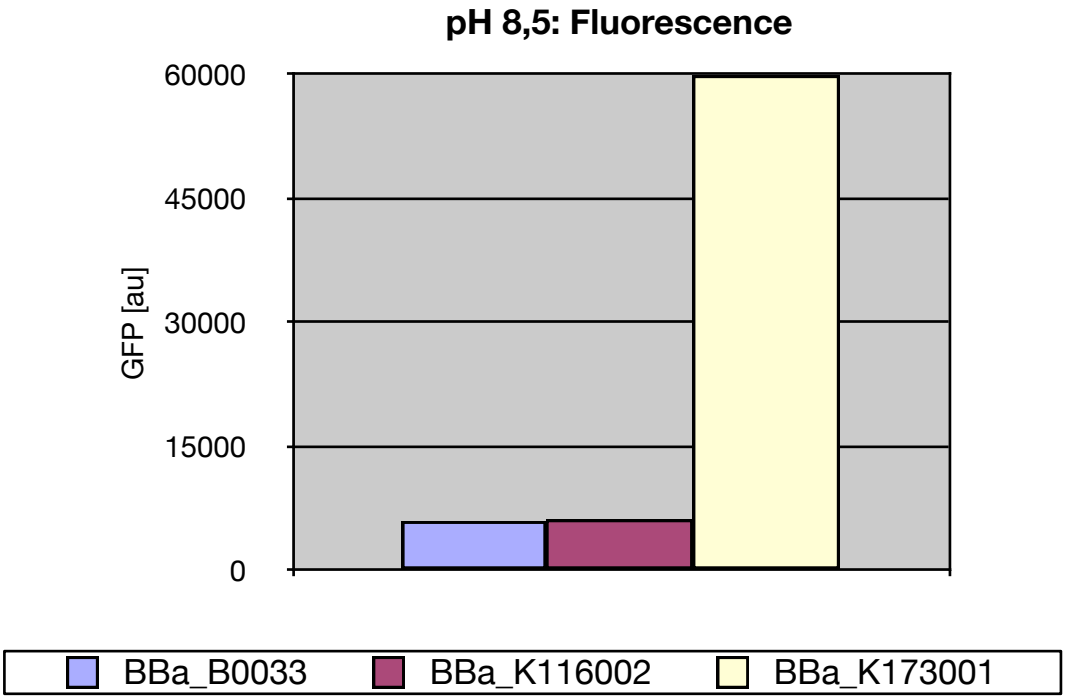


So we decided to measure the fluorescence of the 12 falcon tubes containing LB 171 mM (4 falcon tubes - 4 pH - for each genetic circuit) that have been all the night inside the incubator at 37°C, 220 rpm.




pH 6,6: Fluorescence
60000
45000
30000
15000
0
GFP [au]
BBa_B0033
BBa_K116002
BBa_K173001


pH 7,5: Fluorescence
60000
45000
30000
15000
0
GFP [au]
BBa_B0033
BBa_K116002
BBa_K173001



Comments

No remarkable results this time either. All BBa_K116002 related GFP rate production are still zero as well as negative control BBa_B0033.

Experiment Na+ 250mM

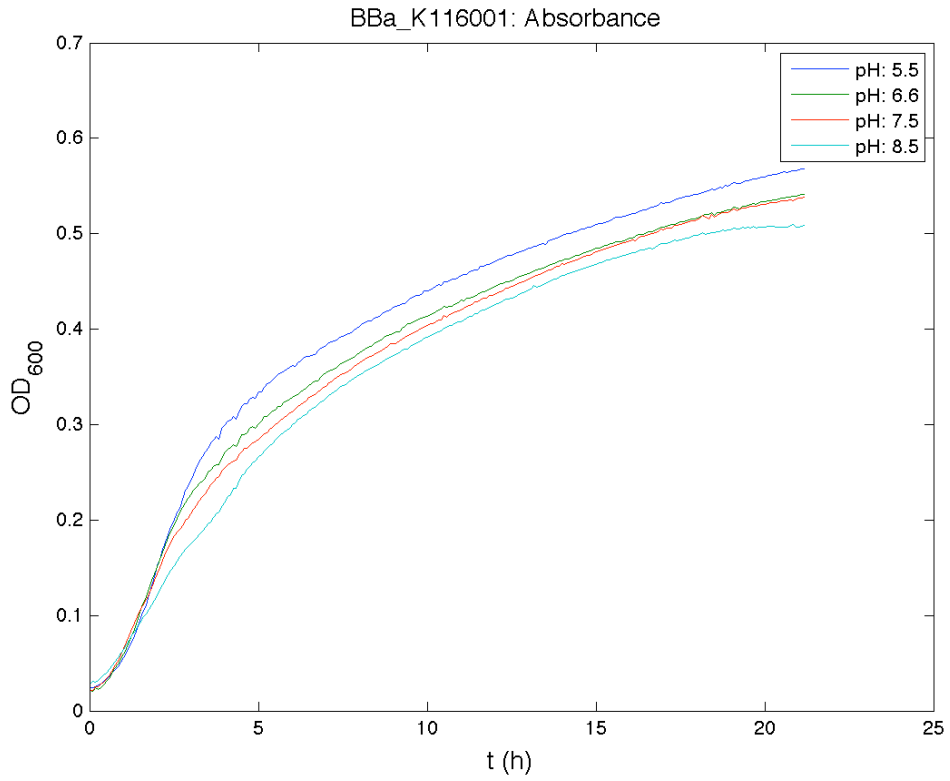
Motivation

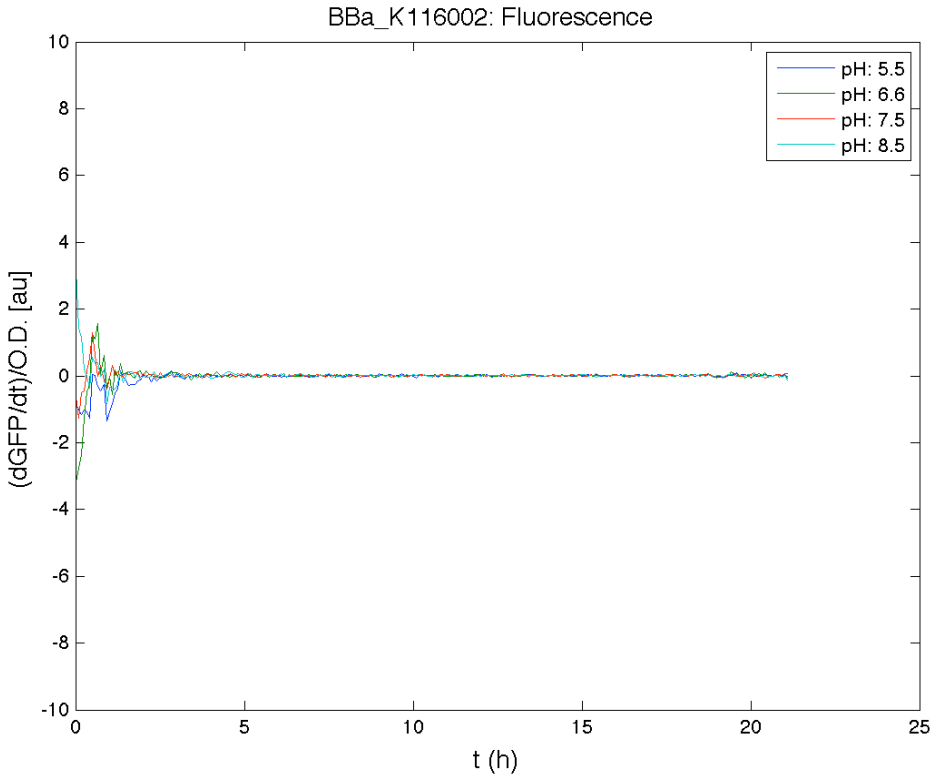
We'll try again to make E. coli producing GFP at the variation of pH in presence of Na+ 250mM.

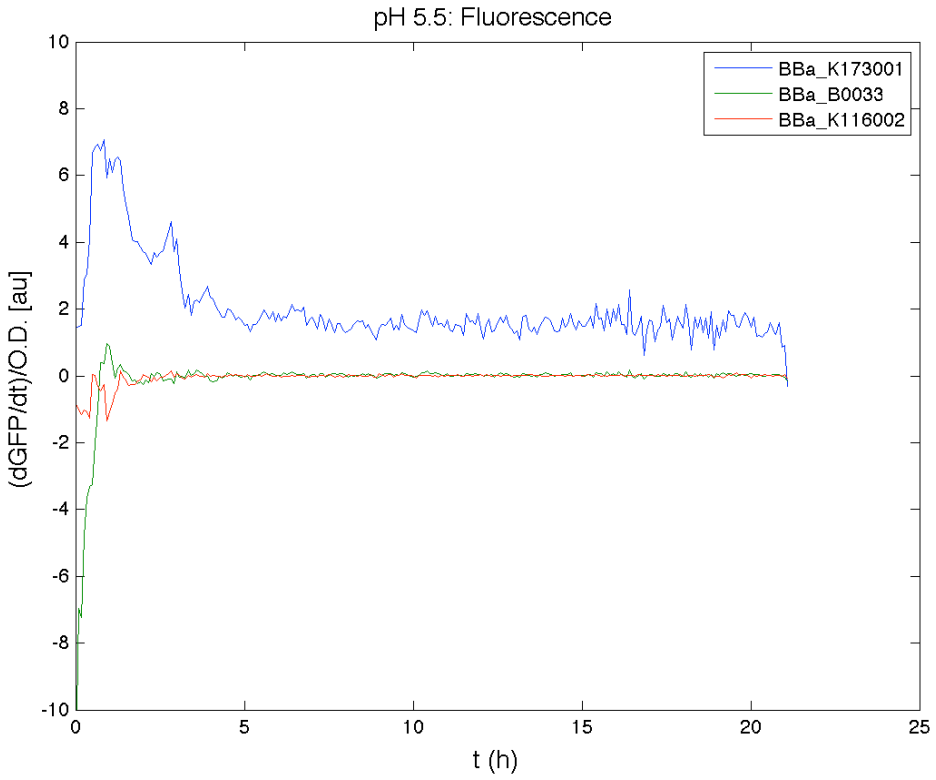
Methods

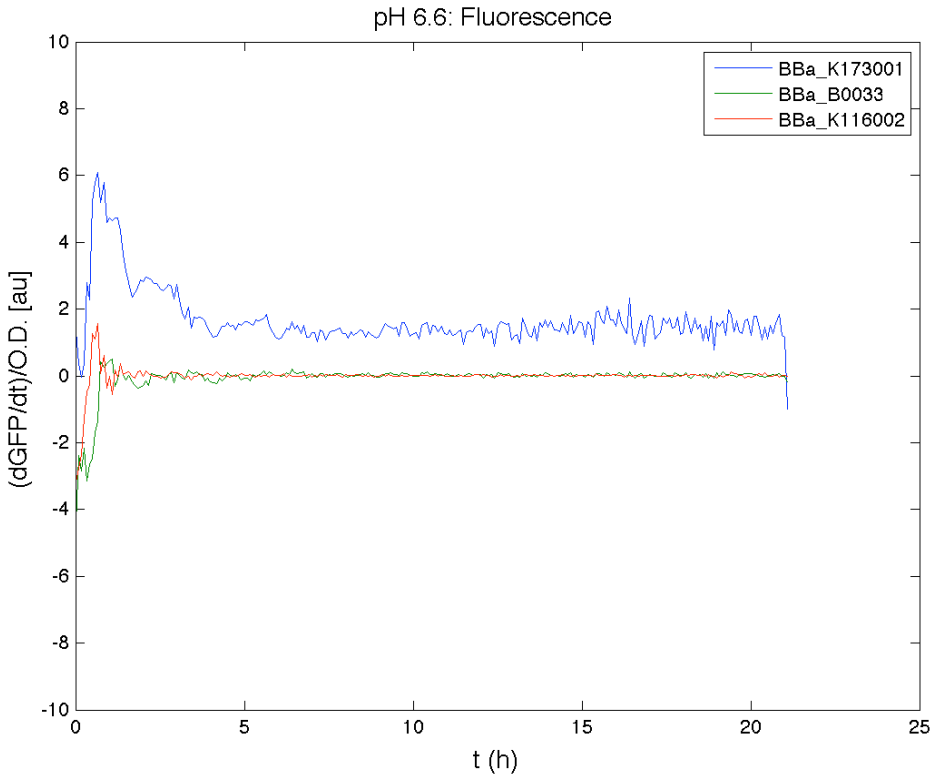
- We prepared falcons of LB NaCl 250mM and adjusted pH using KOH and HCl to values 5.5, 6.6, 7.5 and 8.5.
- We inoculated 8ul of Invitrogen TOP10 containing BBa_K116002 into 4ml of LB + Amp and incubated overnight at 37°C, 220 rpm. We did the same for TOP10 with BBa_K173001 and BBa_B0033 inside.
- Next morning we put 50ul from each of the three falcon into 5ml of LB NaCl 250 mM pH 6.6 and incubated again for five hours and at 37°C, 220 rpm.
- We measured the final O.D. with TECAN Infinite F200 and diluted each genetic circuit into four falcons with LB NaCl 250mM at different pH (5.5 - 6.6 - 7.5 - 8.5) in order to obtain a same O.D. equal to 0,02 (12 falcons overall).
- Then we performed an experiment of 21 hours' duration with measurements of absorbance and fluorescence every 5 minutes with TECAN Infinite F200. Each value is the mean of three measurements and cultures were shaked for 15 seconds every five minutes.

Results




BBa_K116002: Fluorescence
pH: 5.5
pH: 6.6
pH: 7.5
pH: 8.5
(dGFP/dt)/O.D. [au]
t (h)


pH 5.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)


pH 6.6: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)
10
8
6
4
2
0
-2
-4
-6
-8
-10
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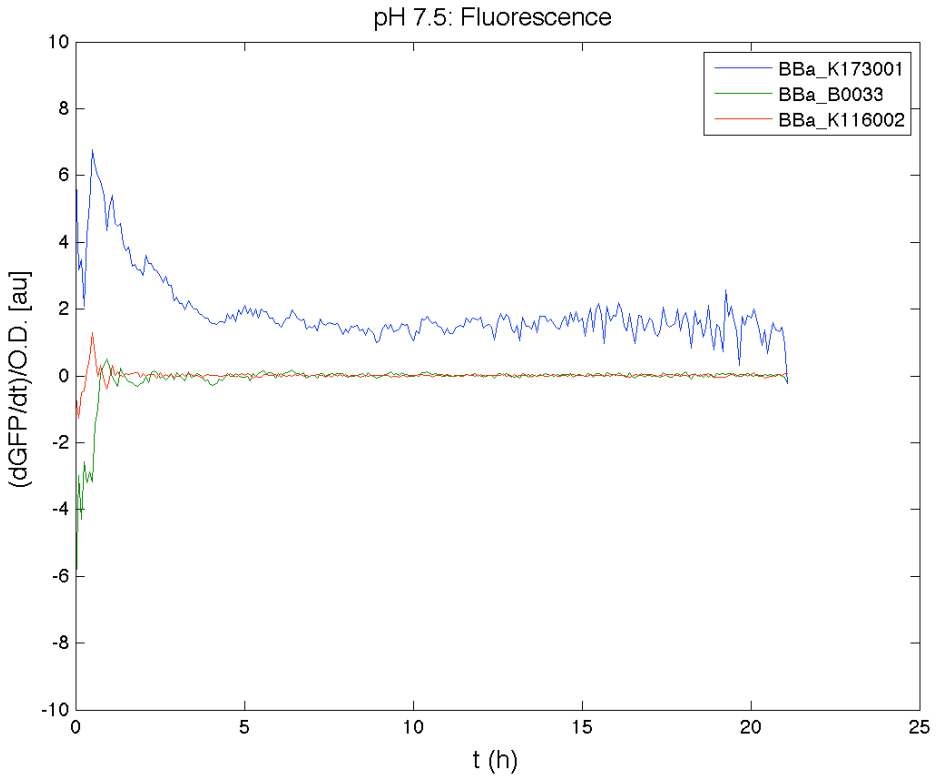
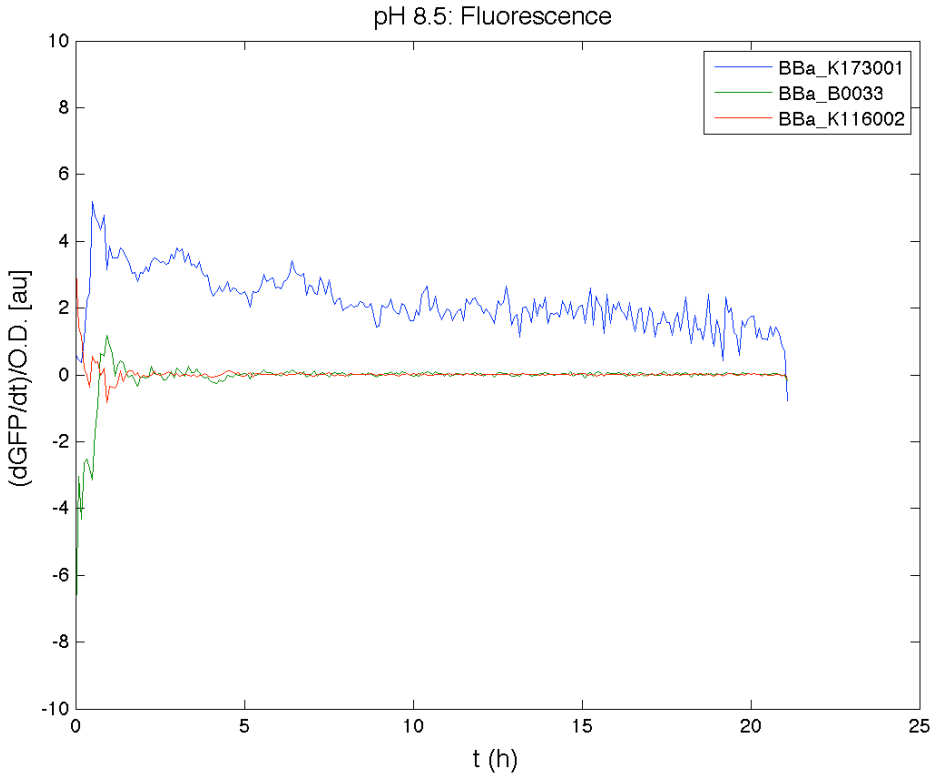
5
10
15
20
25


pH 7.5: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116002
(dGFP/dt)/O.D. [au]
t (h)
10
8
6
4
2
0
-2
-4
-6
-8
-10
0
5
10
15
20
25



Comments

Again BBa_K116002 didn't produce any GFP as well as negative control (BBa_B0033): after the transient due to noise their GFP production rate goes to zero while positive control (BBa_K173001) has a significantly higher one.
After looking better for a motivation in some articles ([Rachel Karpel et al.]) we think this could be because of the E. coli strain: we use TOP10 while a special strain (delta-nhaA) without some membrane proteins that regulate E. coli homeostasis is used in other experiments.

Experiment Na+ 600mM

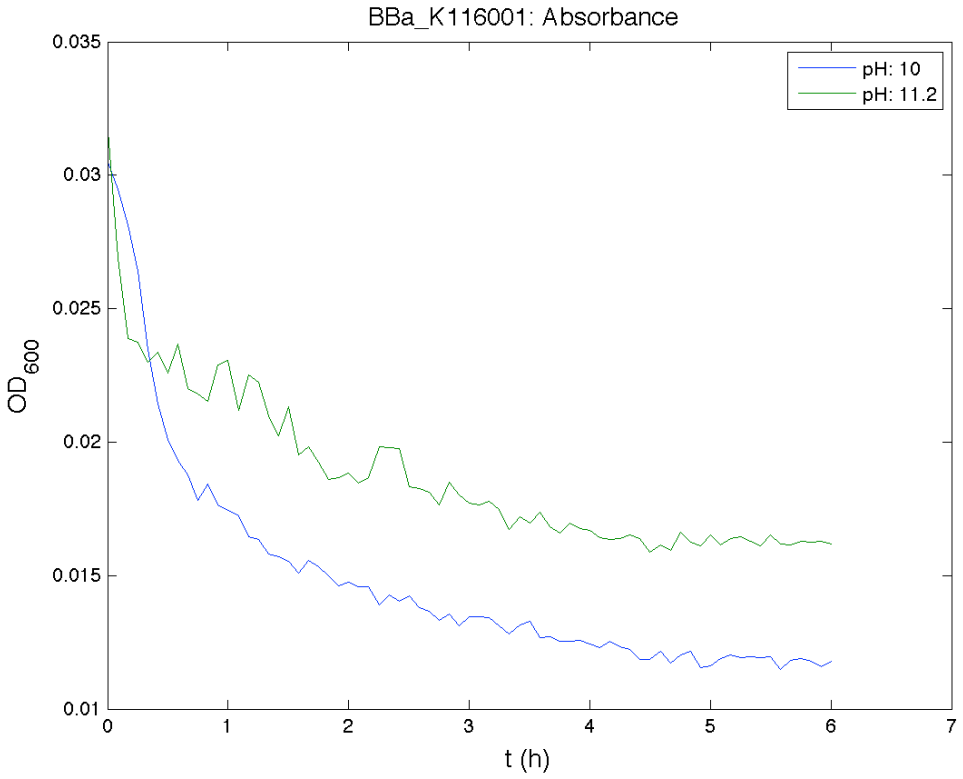
Motivation

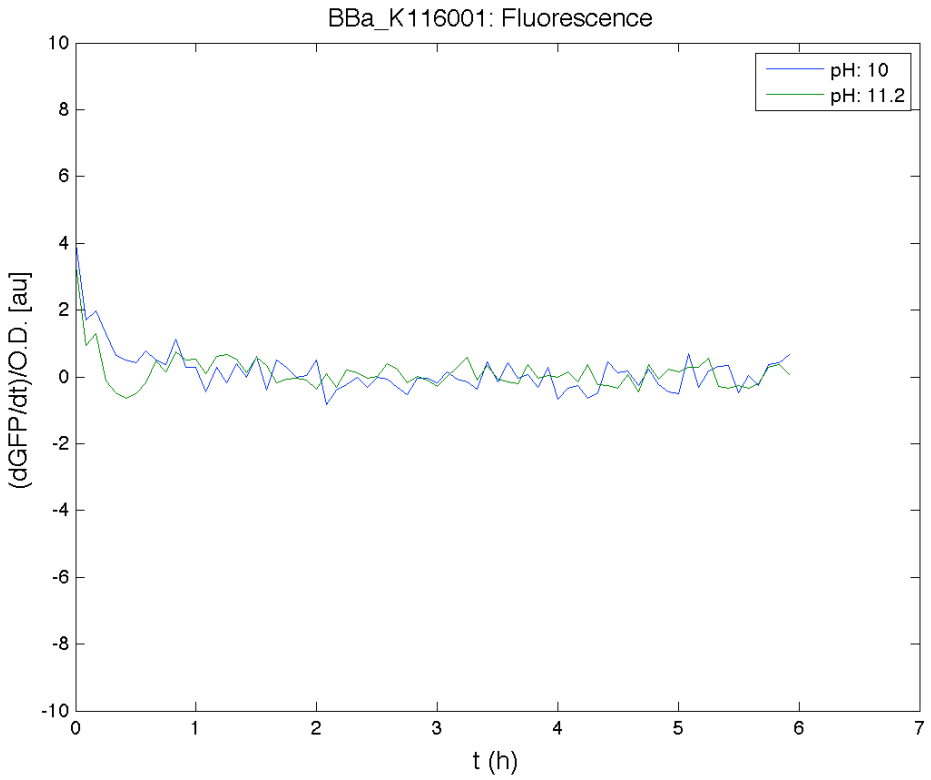
With this last experiment we'll try to give E. coli a sodium (600mM) and pH (10 - 11,2) shock (even if we know it's an hazard) in order to see if BBa_K116002 will produce any significant GFP.

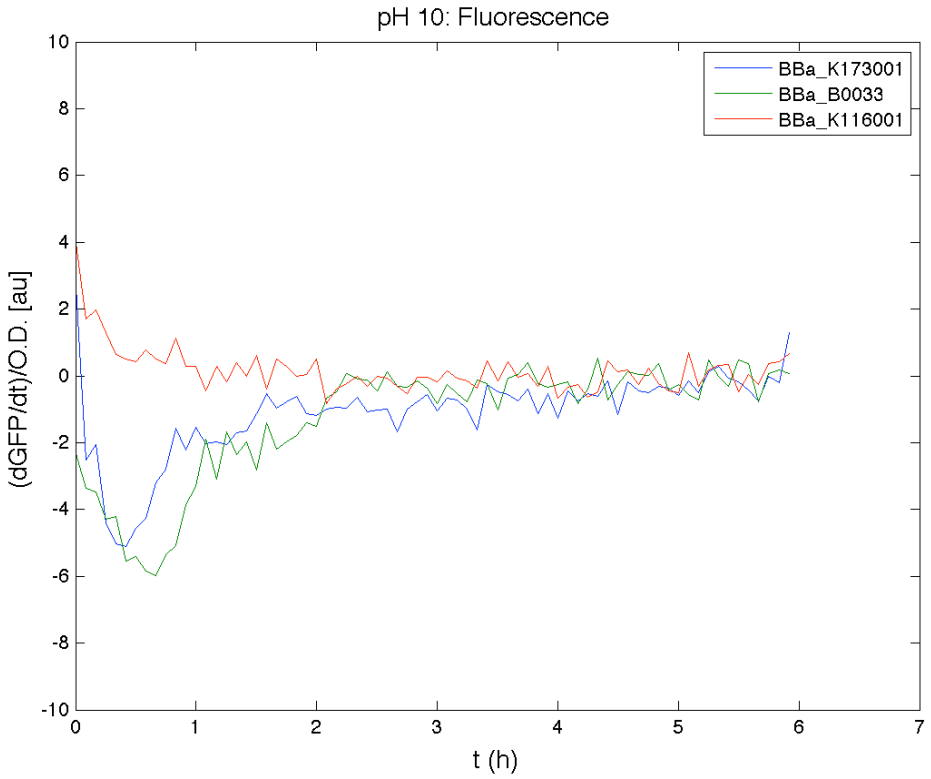
Methods

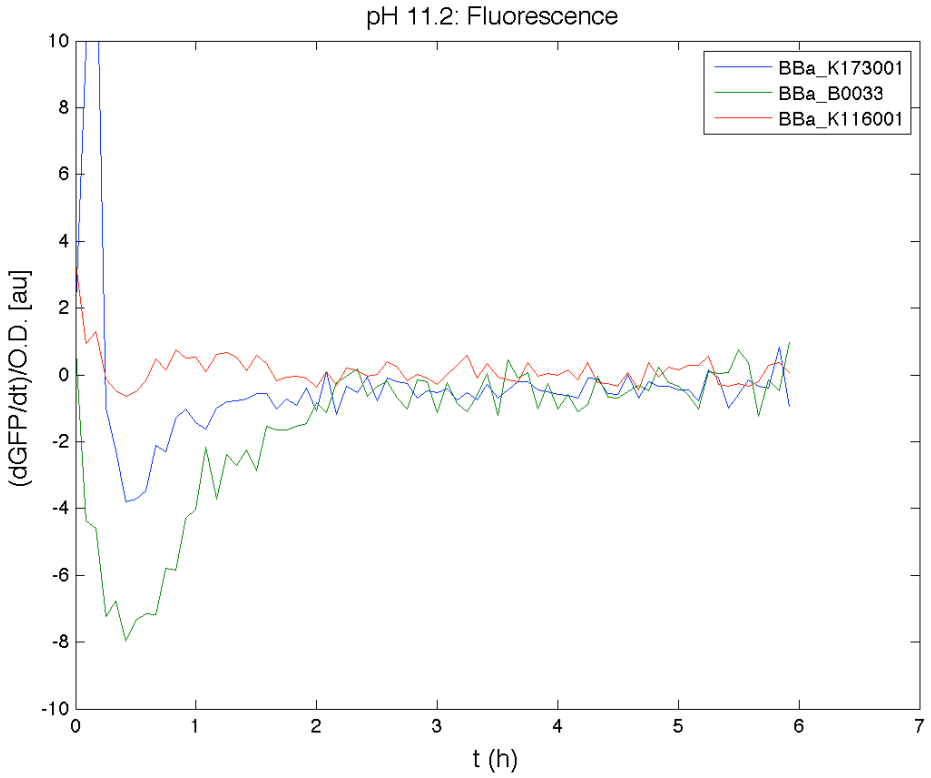
- We prepared LB NaCl 600 mM and adjusted pH using KOH to values 10 and 11,2.
- We inoculated 8ul of Invitrogen TOP10 containing BBa_K116002 into 4ml of LB + Amp and incubated overnight at 37°C, 220 rpm. We did the same for TOP10 with BBa_K173001 and BBa_B0033 inside.
- Next morning we put 50ul from each of the three falcon into 5ml of LB NaCl 70 mM pH 6.6 and incubated again for four hours and a half at 37°C, 220 rpm. After that we measured the final O.D. with TECAN Infinite F200 and diluted each genetic circuit into two falcon tubes with LB NaCl 600mM at different pH (10 - 11.2) in order to obtain a same O.D. equal to 0,02 (6 falcon tubes overall).
- Then we performed an experiment of 6 hours' duration with measurements of absorbance and fluorescence every 5 minutes with TECAN Infinite F200. Each value is the mean of three measurements and cultures were shaked for 15 seconds every five minutes.

Results




BBa_K116001: Fluorescence
pH: 10
pH: 11.2
(dGFP/dt)/O.D. [au]
t (h)


pH 10: Fluorescence
BBa_K173001
BBa_B0033
BBa_K116001
(dGFP/dt)/O.D. [au]
t (h)



Comments

Extreme conditions made all bacteria die, as you can see from growth curves.

Final considerations

In our opinion this sensor (primarily sodium sensor and secondarily pH sensor) needs very particular conditions to work (first of all a specific bacterial strain) we couldn't reproduce, so we consider it almost unusable.

References:

[1] Rachel Karpel, Tamar Alon, Gad GlaserS, Shimon Schuldiner, and Etana Padan - Expression of a Sodium Proton Antiporter (NhaA) in Escherichia coli Is Inducedby Na+ and Li+ Ions - The Journal of Biological Chemistry. 1991 Nov; 266(32):21753-21759.

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