# October

## October 7th

**Experiment:** Lysin induction
**Responsables:** Pablo Delgado
**Protocol code:** InEc
**Protocol modifications or specifications:** The culture was prepared with 10 mL of preculture, 90 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM IPTG was added. It was incubated for 10 hours.
**Results:** SDS-PAGE from october 8th.

## October 7th

**Experiment:** Lysin extraction
**Responsables:** María José
**Protocol code:** ExEc
**Protocol modifications or specifications:** total volume from induction was centrifuged and pellet was resuspended in 10 mL of Lysis Buffer. Then, 80 uL of PMSF, 0.5 uL of DNase, 200 uL of MgCl2 and 0.6 uL of mercaptoethanol were added.
**Results:** SDS-PAGE from October 8th.

## October 7th

**Experiment:** Lysin purification
**Responsables:** María José
**Protocol code:** LysP
**Protocol modifications or specifications:** Supernatant from protein extraction was incubated overnight with 100 uL of resin, previously washed. Resin was resuspended in 10 mM Imidazole.
**Results:** SDS-PAGE from october 8th.

## October 8th

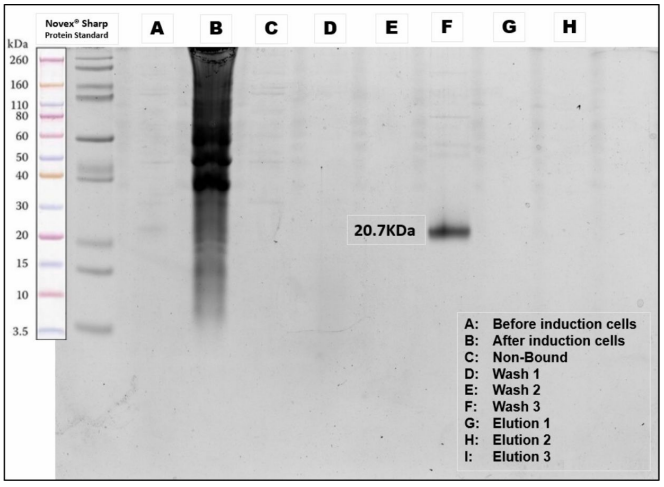
**Experiment:** Lysin purification
**Responsables:** María José
**Protocol code:** LysP
**Protocol modifications or specifications:** After incubation, the protein was purified by affinity chromatography. Non Bound, Wash 1 with 10 mM Imidazole, Wash 2 with 20 mM Imidazole and three Elutions with 500 mM Imidazole were obtained.
**Results:** SDS-PAGE from october 8th.

## October 8th

**Experiment:** SDS-PAGE
**Responsables:** María José
**Protocol code:** SDSPAGE

**Protocol modifications or specifications:** SDS-PAGE was run with samples of lysin induction, extraction and purification from October 7th. The gel was run for 30 min at 90 V and 1 hour and a half at 120 V.

**Results:**




## October 9th

**Experiment:** Lysin induction

**Responsables:** Pablo Delgado

**Protocol code:** InEc

**Protocol modifications or specifications:** The culture was prepared with 25 mL of preculture, 375 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM IPTG was added. It was incubated for 6 hours.

**Results:** SDS-PAGE from October 10th.

## October 9th

**Experiment:** Lysin extraction

**Responsables:** María José

**Protocol code:** ExEc

**Protocol modifications or specifications:** total volume from induction was centrifuge and then, the eight pellets were resuspended in 5 mL of Lysis Buffer. Resuspended pellets were joined in two falcons (20 mL each one) and then 200 uL of PMSF, 1 uL of DNase, 400 uL of MgCl2 and 1.7 uL of mercaptoethanol were added.

**Results:** SDS-PAGE from October 10th.

## October 10th

**Experiment:** Lysin purification

**Responsables:** María José

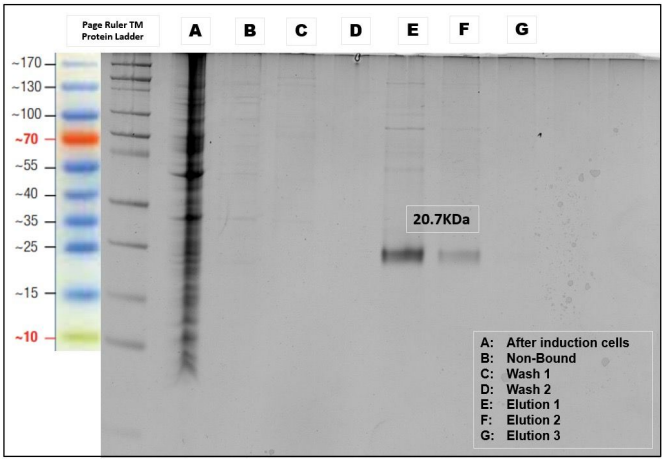
**Protocol code:** LysP

**Protocol modifications or specifications:** Supernatant from protein extraction was incubated overnight with 400 uL of resin, previously washed. Resin was resuspended in 10 mM Imidazole. After incubation, the protein was purified by affinity chromatography. Non Bound, Wash 1 with 10 mM Imidazole, Wash 2 with 20 mM Imidazole and three Elutions with 500 mM Imidazole were obtained.

**Results:** SDS-PAGE from October 10th.

## October 10th

**Experiment:** SDS-PAGE
**Responsables:** María José
**Protocol code:** SDSPAGE
**Protocol modifications or specifications:** SDS-PAGE was run with samples of lysin induction, extraction and purification from October 7th. The gel was run 30 min at 90 V and 1 hour and a half at 120 V.
**Results:**




## October 11th

**Experiment:** Plasmid Isolation
**Responsables:** Pablo Delgado
**Protocol code:** ThermoScientific Kit, K0503
**Protocol modifications or specifications:** At the final step, plasmidial DNA was eluted using 40µL of nuclease free water.
**Results:**

| Name | Resistance | Concentration (ng/uL) | Absorbance |
|---|---|---|---|
| **ILC1** | C | 231.5 | 1.85 |
| **ILC2** | C | 218 | 1.85 |
| **ILC3** | C | 204.6 | 1.84 |
| **ILC C+** | C | 172.5 | 1.85 |
| **ILC C-** | C | 99.6 | 1.82 |
| **Lisina E. coli** | A | 415 | 1.87 |
| **K2235000** | A | 430 | 1.86 |

## October 11th

**Experiment:** Lysin dialysis and concentration
**Responsables:** María José
**Protocol code:** Protein_DC

**Protocol modifications or specifications:** Lysin was dialysed and concentrated with buffer 50 mM Tris pH 7.45 in a 3K Amicon. It was centrifuged at 5000 g for approximately 1 hour/round.
**Results:**

| Concentration | |
|---|---|
| **Lysin*** | 0.12 mg/mL |
| **Imidazole** | 0.59 mM |

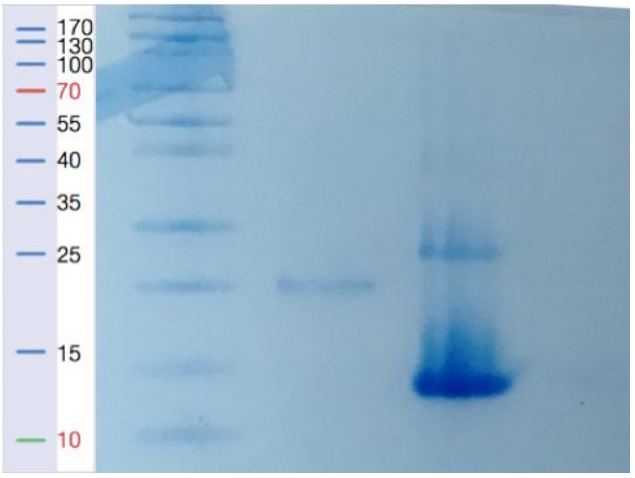
*Protein correction factor was 0.659

## October 11th

**Experiment:** *Clostridium difficile* NTCC 13307 culture
**Responsables:** Anthony Mora
**Protocol code:** a cryopreserved culture was stroke in Blood agar.
**Protocol modifications or specifications:** None
**Results:** Not needed

## October 12th

**Experiment:** SDS-PAGE
**Responsables:** María José
**Protocol code:** SDSPAGE
**Protocol modifications or specifications:** SDS-PAGE was run with samples of lysin dialysis and concentration from October 11th. The gel was run 30 min at 90 V and 1 hour and a half at 120 V.
**Results:**




(1) Molecular weight marker. (2) Lysin dialysed and concentrated. (3) Lysozyme 0.5 mg/mL.

## October 12th

**Experiment:** AIP induction
**Responsables:** María José Durán
**Protocol code:** InEc
**Protocol modifications or specifications:** The culture was prepared with 40 mL of preculture, 360 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM IPTG was added. It was incubated O/N.
**Results:** SDS-PAGE from October 15th.

## October 13th

**Experiment:** AIP extraction
**Responsables:** María José
**Protocol code:** ExEc
**Protocol modifications or specifications:** total volume from induction was centrifuge and then, the eight pellets were resuspended in 5 mL of Lysis Buffer. Resuspended pellets were joined in two falcons (20 mL each one) and then 200 uL of PMSF, 1 uL of DNase, 400 uL of MgCl2 and 1.7 uL of mercaptoethanol were added.
**Results:** SDS-PAGE from October 15th.

## October 13th

**Experiment:** AIP concentration
**Responsables:** María José
**Protocol code:** Protein_DC
**Protocol modifications or specifications:** AIP was dialysed and concentrated with buffer 50 mM Tris pH 7.45 in a 3K Amicon. It was centrifuged at 5000 g for approximately 1 hour/round.
**Results:** SDS-PAGE from October 15th.

## October 13th

**Experiment:** Lysin induction
**Responsables:** María José
**Protocol code:** InEc
**Protocol modifications or specifications:** The culture was prepared with 40 mL of preculture, 360 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM IPTG was added. It was incubated for 6 hours.
**Results:** SDS-PAGE from October 13th.

## October 13th

**Experiment:** Lysin extraction
**Responsables:** María José
**Protocol code:** ExEc
**Protocol modifications or specifications:** total volume from induction was centrifuged and then, the eight pellets were resuspended in 5 mL of Lysis Buffer. Resuspended pellets were joined in two falcons (20 mL each one) and then 200 uL of PMSF, 1 uL of DNase, 400 uL of MgCl2 and 1.7 uL of mercaptoethanol were added.
**Results:** SDS-PAGE from October 13th.

## October 13th

**Experiment:** Lysin purification
**Responsables:** María José
**Protocol code:** LysP
**Protocol modifications or specifications:** Supernatant from protein extraction was incubated overnight with 400 uL of resin, previously washed. Resin was resuspended in 10 mM Imidazole. After

incubation, the protein was purified by affinity chromatography. Non Bound, Wash 1 with 10 mM Imidazole, Wash 2 with 20 mM Imidazole and three Elutions with 500 mM Imidazole were obtained.
**Results:** SDS-PAGE from October 13th.

## October 13th

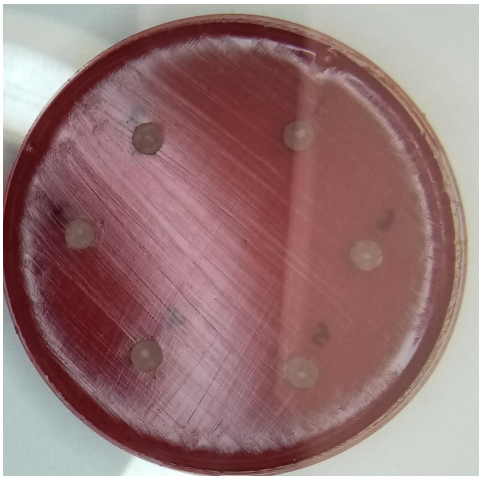
**Experiment:** Lysin dialysis and concentration
**Responsables:** María José
**Protocol code:** Protein_DC
**Protocol modifications or specifications:** Lysin was dialysed and concentrated with buffer 50 mM Tris pH 7.45 in a 3K Amicon. It was centrifuged at 5000 g for approximately 1 hour/round.
**Results:**

| Concentration | |
|---|---|
| **Lysin*** | - |
| **Imidazole** | 11.9 mM |

*Protein correction factor was 0.659

## October 14th

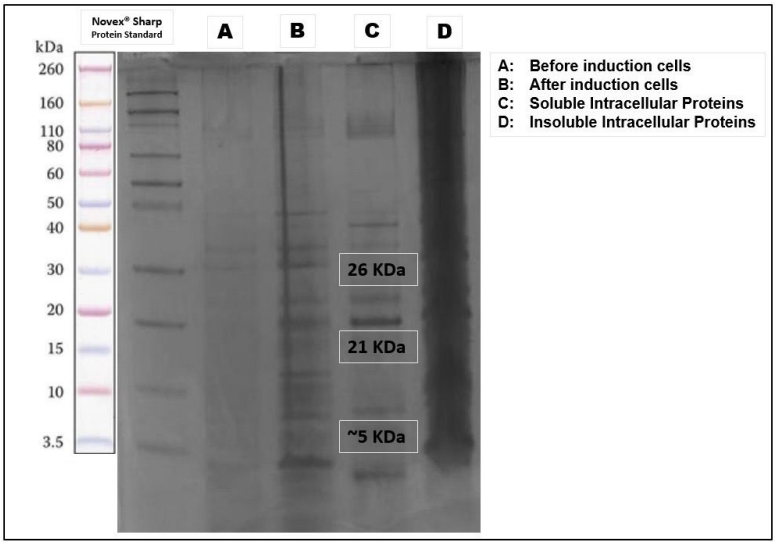
**Experiment:** Lysine Susceptibility Testing on *Clostridium difficile NTCC 13307*
**Responsables:** Anthony Mora
**Protocol code:** Lys_Sus
**Protocol modifications or specifications:** PBS was used as a negative control
**Results:** There was no protein activity, no inhibition halos were observed



Columbia agar with *C. difficile* NTCC 13307 inhibition zones

## October 15th

**Experiment:** SDS-PAGE
**Responsables:** María José
**Protocol code:** SDSPAGE
**Protocol modifications or specifications:** SDS-PAGE was run with the dialysed AIP supernatant extract from October 13th. The gel was run for 30 min at 90 V and 1 hour and a half at 120 V.
**Results:**




## October 16th

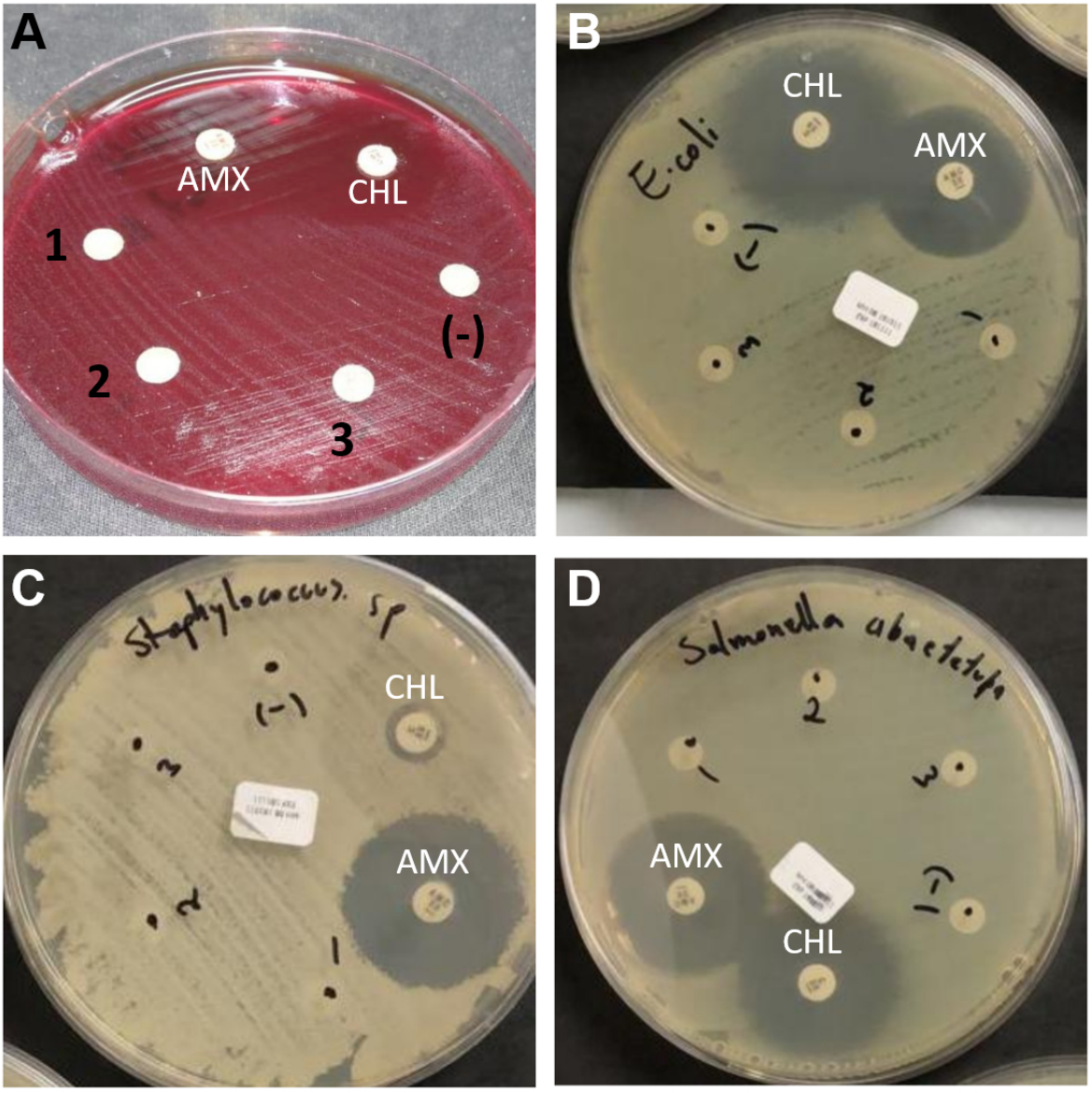
**Experiment:** AIP induction
**Responsables:** María José Durán
**Protocol code:** InEc
**Protocol modifications or specifications:** The culture was prepared with 7.5 mL of preculture, 67.5 mL of LB medium and 100 mg/mL of ampicillin. At the induction step, 1 mM IPTG was added. It was incubated for 9 hours. After centrifugation, supernatant was stored at 4°C for a later lyophilization.
**Results:** SDS-PAGE from October 19th.

## October 17th

**Experiment:** AIP extraction
**Responsables:** María José
**Protocol code:** ExEc
**Protocol modifications or specifications:** total volume from induction was centrifuged and then, the eight pellets were resuspended in 5 mL of Lysis Buffer. Resuspended pellets were joined in two falcons (20 mL each one) and then 200 uL of PMSF, 1 uL of DNase, 400 uL of MgCl2 and 1.7 uL of mercaptoethanol were added.
**Results:** SDS-PAGE from October 19th.

## October 17th

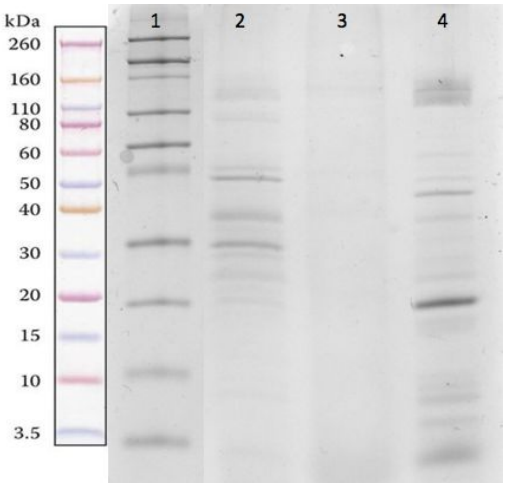
**Experiment:** Lysin Susceptibility Testing on *Clostridium difficile NTCC 13307*
**Responsables:** Anthony Mora
**Protocol code:** Lys_Sus
**Protocol modifications or specifications:** *Salmonella abaetetuba, Escherichia coli and Staphylococcus aureus* were tested in Mueller-Hinton agar with amoxicillin and chloramphenicol as positive controls and PBS as negative control. The inoculum was standardized to McFarland 0.5.
**Results:** There was no protein activity, no inhibitory halos were observed around the disks with protein (1, 2 and 3)




Lysis assay of endolysin CD27L1-179 on: A) *Clostridium difficile*, B) *Escherichia coli*, C) *Staphylococcus sp.* and D) *Salmonella abaetetuba*. Amoxicillin (AMX) and chloramphenicol (CHL) were tested as positive control agents and PBS as negative control (-); both antibiotics were used by recommendation of Andino-Molina and colleagues (2019). Lysin was assayed with three different concentrations (1: 120µg/mL, 2: 60µg/mL and 3: 30µg/mL) to evaluate its growth inhibition capacity.

## October 19th

**Experiment:** SDS-PAGE
**Responsables:** María José
**Protocol code:** SDSPAGE
**Protocol modifications or specifications:** SDS-PAGE was run with the dialysed AIP supernatant from induction and extraction, and lyophilized induction supernant from October 13th. The gel was run for 30 min at 90 V and 1 hour and a half at 120 V.
**Results:**



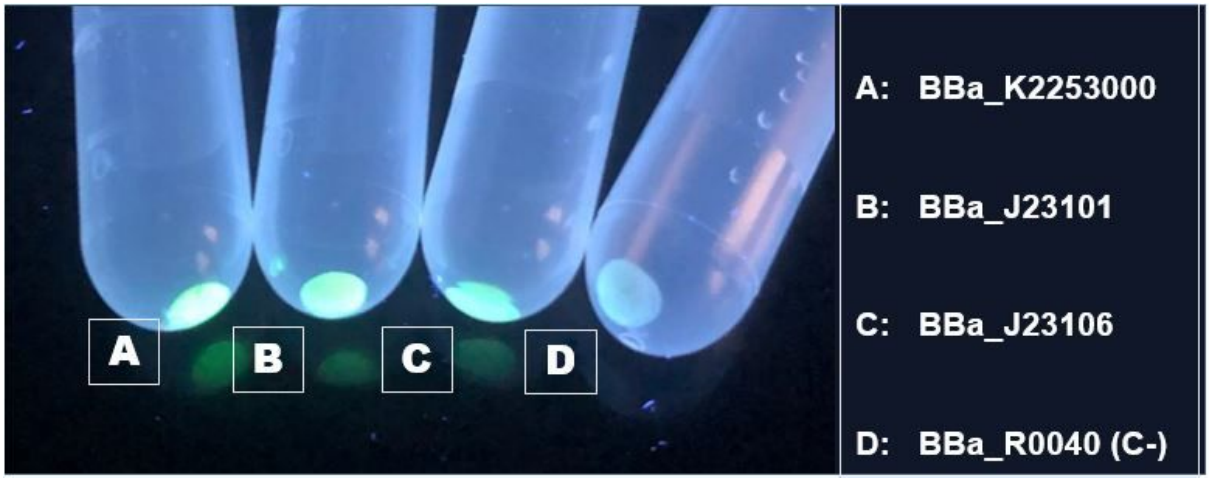

(1) Molecular Marker, (2) Extracell proteins after dialyzation, (3) Extracell proteins after lyophilization, (4) Soluble Intracell proteins.

## October 20th

**Experiment:** Quantification of bacterial fluorescence using independent calibrants for promotor K225300 characterization.
**Responsables:** Anthony Mora, Paula Thiel, Pablo Delgado and Diego Rojas
**Protocol code:** Quan_fluo
**Protocol modifications or specifications:** The device 3: J23117.B0034.E0040.B0015 in pSB1C3 was no tested in the experiment
**Results:**

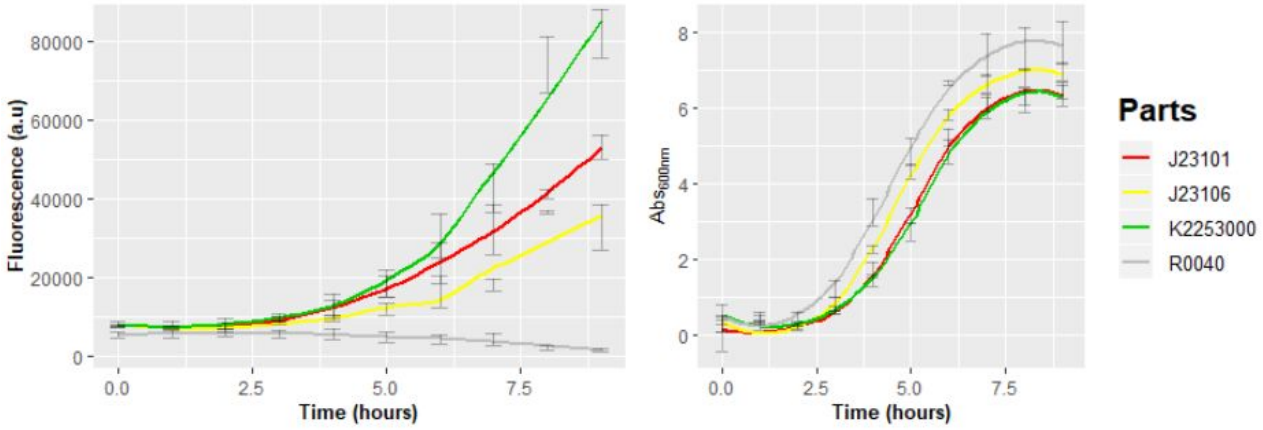
In order to measure the expression strength of this part from Lactococcus in *E. coli* we placed a GFP and a terminator downstream of this sequence. The final construct designed for this characterization consisted on the following iGEM parts: K2253000+E0040+B0015.

Therefore, we used two other GFP expression cassettes (provided by iGEM 2016 InterLab), which were regulated under a well characterized Anderson Promoter and an Elowitz RBS (B0034), as references for comparison. The strong expression cassette contained the J23101 promoter and the medium expression cassette the J23106 promoter. Both of this cassettes were cloned in the same iGEM backbone as the K2253000 characterization construct.




Qualitative differences between this part and standard biobricks for InterLab iGEM2016

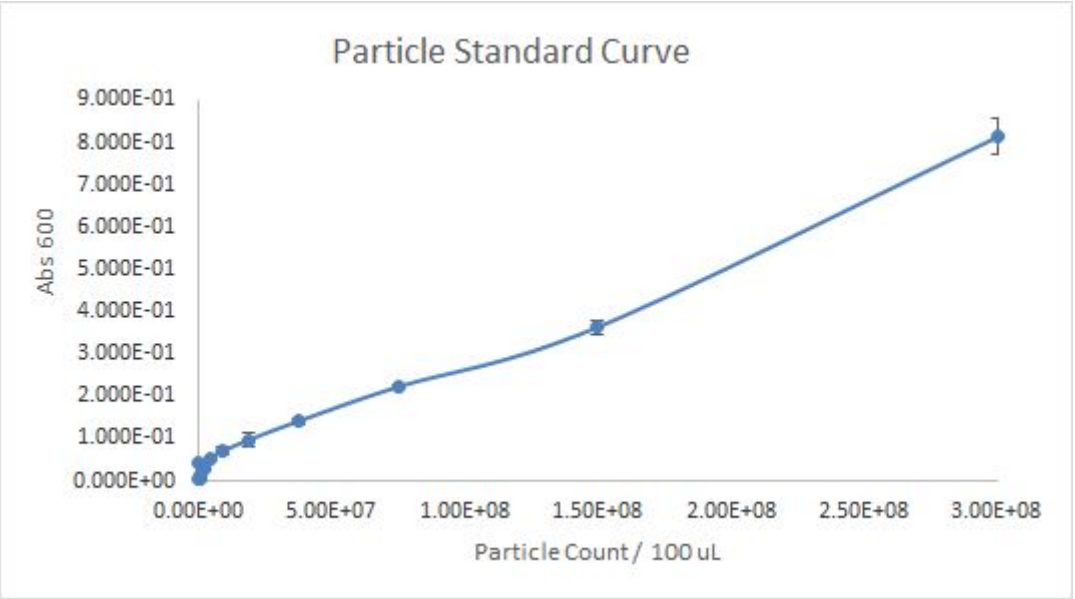
The curve was started with an $OD_{600}$ 0.05. This was done in triplicate. It was incubated for 9 hours at 37°C and 200 rpm. Samples for GFP (485nm, 528nm) and $OD_{600}$ quantification were taken every hour and measured in a Tecan Biotek plate reader Non paremetrics statics analysis were implemented with Minitab 19 Statistical Software (2019).



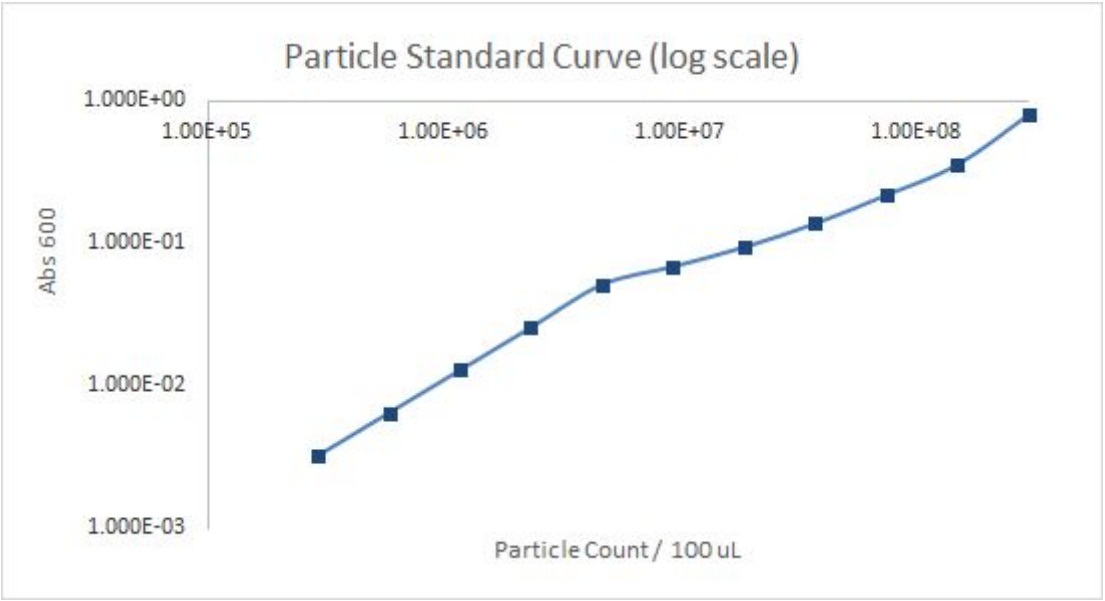

Growth curve and fluorescence measurement of K2253000 promoter with GFP

## October 21st

**Experiment:** iGEM 2019 Plate Reader Abs600 (OD) Calibration
**Responsables:** Diego Rojas
**Protocol code:** none
**Protocol modifications or specifications:** none
**Results:**
After following the iGEM 2019 calibration protocol we got a Mean particles/Abs600 value of $3.90x10^8$ for Mean of med-high levels to standardized our experiments and OD600 measurements with a $R^2$ correlation value of 0.9909 in our Biotek Synergy HTX Multi-mode microplate reader.




Silica Beads Particles Standard Curve Calibration

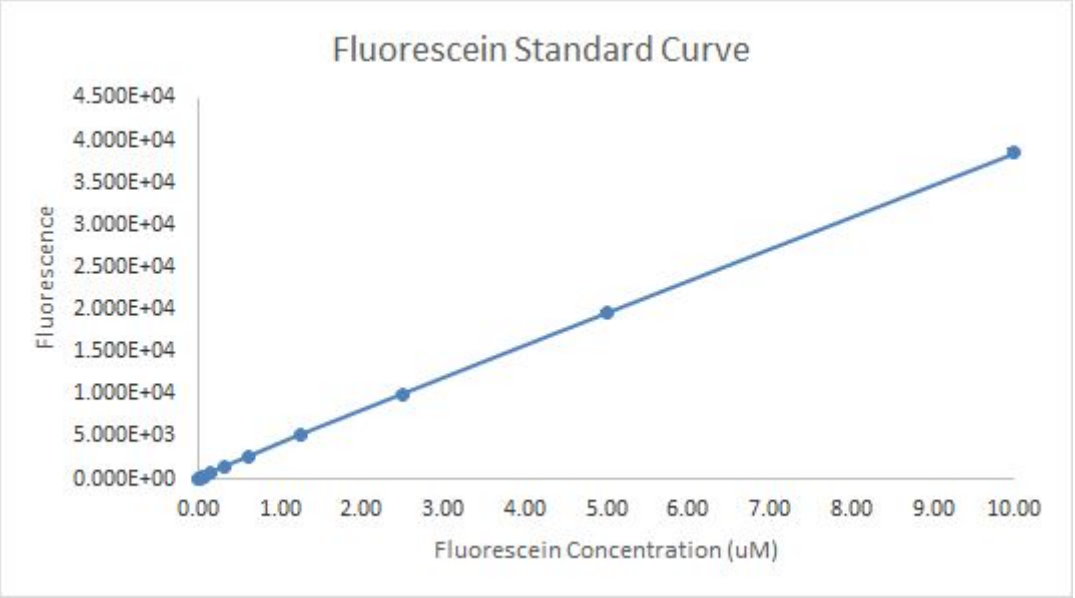



Silica Beads Particles Standard Curve Calibration at log scale

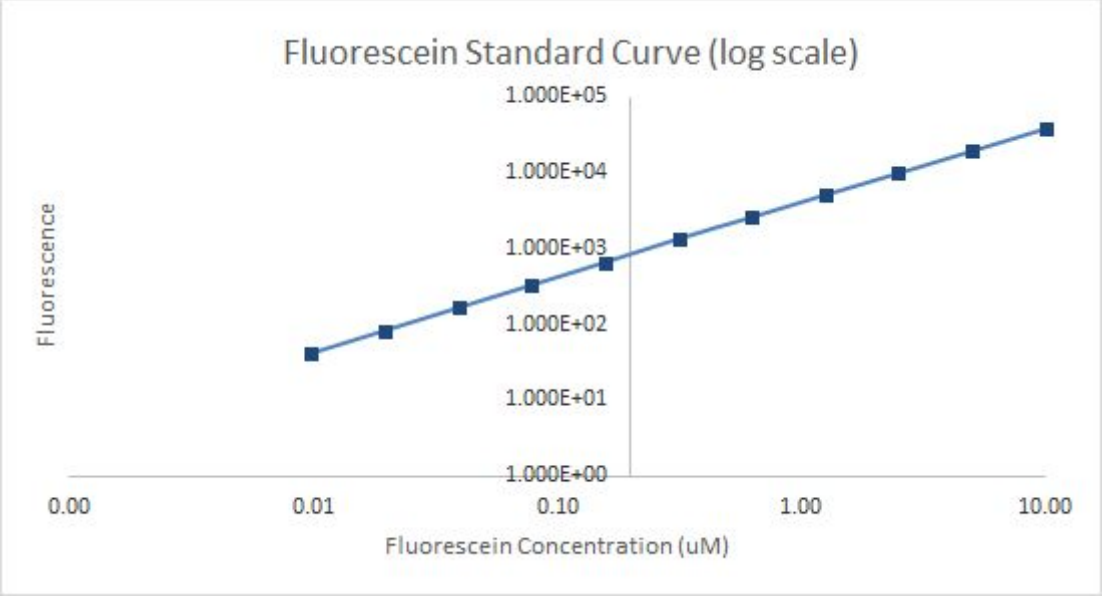
## October 21st

**Experiment:** iGEM 2019 Plate Reader Fluorescence Calibration
**Responsables:** Diego Rojas
**Protocol code:** none
**Protocol modifications or specifications:** none
**Results:**

After following the iGEM 2019 calibration protocol we got a MEFL/a.u. value of $1.47x10^{10}$ to standardized our experiments and fluorescence measurements with a $R^2$ correlation value of 0.9999 in our Biotek Synergy HTX Multi-mode microplate reader.




Fluorescein Standard Curve Calibration




Fluorescein Standard Curve Calibration at log scale