MATERIALS AND METHODS

Biobicks

The following bioparts were recuperated from the 2009 and 2012 iGEM distribution plates

Table 1. Characteristics of the used bioparts in the promotor characterization

Biopart	Characteristic
J23101	Constitutive promoter
J23102	Constitutive promoter
J23104	Constitutive promoter
J23107	Constitutive promoter
J23108	Constitutive promoter
J23111	Constitutive promoter
J23115	Constitutive promoter
J54103	Promotor-GFP
J04450	RFP Coding Device

Circuits construction

Plasmids were obtained by miniprep technique using the kit: Zyppy Plasmid Miniprep Kit **(PROTOCOL *E.coli* MINIPRERP KIT)** and quantified by Nanodrop

For the constitutive promoters (J23101, J23102, J23104, J23107, J23108, J23111 y J23115) the following digestions were used: 8 hours at 37ºC wit a final volume of 30 µL:

Tabla 2. Used quantitles for double digestions

Reactive	Quantity (µL)
Buffer (Tango 1X)	3
Enzyme (PstI)	1
Enzyme (SpeI)	2
Plasmid	2
Water	22

After making the digestions, a gel was made to confirm the fragments released and the band purification was performed using the kit: Gel DNA Recovery Kit **(E. coli RECOVERY PROTOCOL DNA KIT).**

The biopart J54103 was extracted from the plate and amplified by PCR reaction of 50 µl, 25 cycles of 94 ° C 0:30, 0:45 55 ° C, 72 ° C 1:15, 72 ° C 8:00 and 4 ° C Hold Infinite

Table 3. Used quantities for amplifying

Reactive	Quantity (µL)
Water	36.3
Buffer	5
$MgCl_2$	4
Taq	1.2
FWD	1
REV	1
dNTP´s	10
Plasmid	0.5

Following the amplification of said biopart, a digestion was made for 8 hours at 37 ° C

Tabla 4. Used quantitles for double digestions

Reactive	Quantity (µL)
Buffer (Tango 1X)	3
Enzyme (PstI)	1
Enzyme (SpeI)	2
Plasmid	2
Water	22

Once obtained the said promoters and GFP, each one was ligated to a GFP reporter gene, by ligation of 16 hours at 16 ° C, with a final volume of 20 µL

Tabla 5 Used quantities for the ligation of the promoters wit the GFP

Reactive	Quantity (µL)
Insert (Promotor)	10
Vector (GFP)	1.5
Buffer (Ligase)	2
Enzyme (Ligase T4)	1
Water	5.5

Following the ligation of 16 hours, chemiocompetents TOP 10 cells were transformed by heat shock. At 12 hours of culture, the colonies were observed, the ones that were selected were those which showed green fluorescence under UV light.
Miniprep was performed to extract the plasmid of selected cells, as well as the vector J04450 cells. They were digested with the enzymes EcoRI and PstI for 16 hours.
The bands were purified cutting the inserts constructions of promoters and the J04450 biopart containing the chloramphenicol resistance.
Following this, a ligation of the promoter and the vector was made.
From colonies which showed fluorescence, a final confirmation was performed by a digestion of the extracted DNA plasmid.

Promotors Characterization

With the selected colonies, an overnight culture was made in M9 media(minimal media supplemented with 0.2% CAA). After 12 hours the culture was transferred to a 96 well plate at a 1:10 dilution (20 µl of culture and 180 µL of fresh M9 medium).

OD and fluorescence measurements of the selected colonies were performed at intervals of 30 minutes for 16 h.
From the results the PopS were calculated (polymerases per second)