Our team has contributed to **'SECOND INTERNATIONAL INTERLAB MEASUREMENT STUDY'**. Along with this, the results of measurements for the devices which we constructed are shared below.

The interlab study aims at understanding the comparative efficiency of promoter picked from a family of constitutive promoters in expressing a GFP construct present downstream to it. The fluorescence data from these constructs were to be submitted.

The strain which was used as a host for the expression of all these constructs is DH5α from *Escherichia coli*. All the three devices were cloned in a high copy pSB1C3 vector backbone, using 3A Assembly protocol, given in the iGEM Protocol Book. They were grown in LB with Chloramphenicol.

EXPERIMENTAL DESIGN:

We used the **3A-Assembly** protocol to assemble our devices. The GFP gene needed to be inserted downstream of the three constitutive promoters provided.

The promoters were digested using enzymes **EcoRI** and **SpeI**.
The GFP biobrick was digested using enzymes **XbaI** and **PstI**.
The linearized plasmid backbone was digested using enzymes **EcoRI**, **PstI** and **DpnI**.

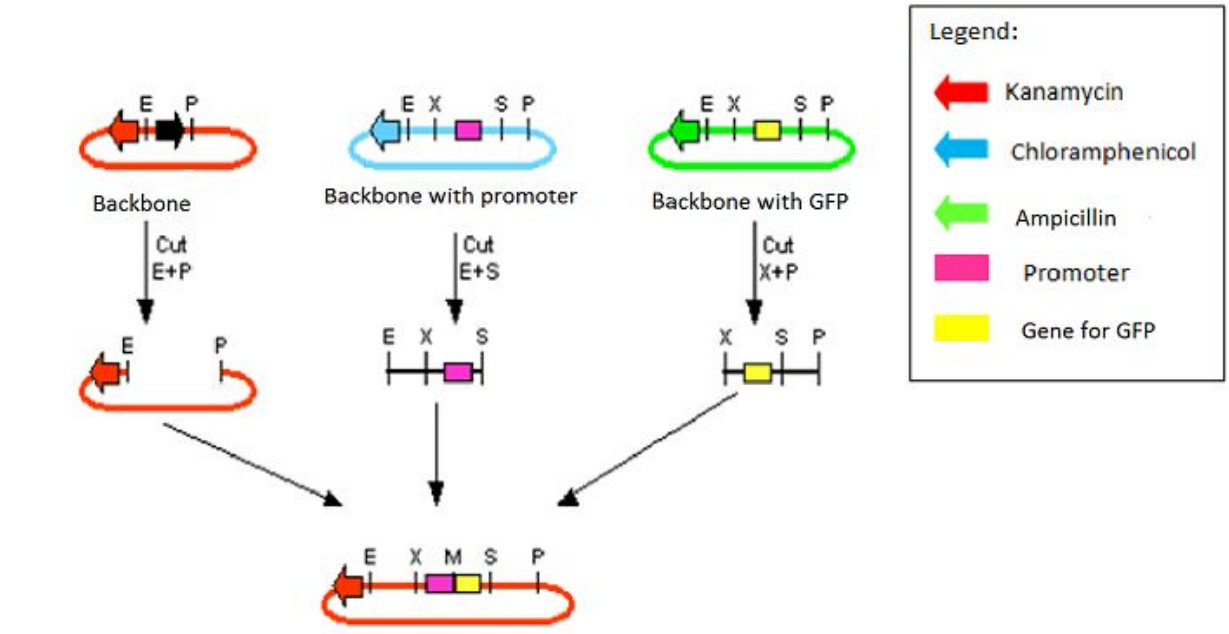

Fig 5: 3A assembly: Strategy with using different antibiotics for cloning the promoter and gene of interest from their respective plasmid backbones (in blue and green) into a linearised backbone (in red)

DpnI is used to digest any template DNA from production.

Since the promoters were in the pSB1C3 backbone which had the gene coding for resistance to chloramphenicol, and the GFP gene was in the pSB1A2 backbone which had the gene coding for resistance to ampicillin, we initially assembled the devices using the pSB1K3 (kanamycin) linearized backbone.

We then set up a **2A-Assembly** to cut out the Promoter+GFP from the pSB1K3 backbone, and insert it in the pSB1C3 backbone. Our final construct consisted of the promoter upstream of the GFP in the pSB1C3 backbone.The fluorescence measurements were made in this construct.

MATERIALS AND METHODS:

Biobrick Parts:

Part Number	Location in Kit	Backbone
J23101 (Promoter1)	Plate1, Well 20K	Chloramphenicol
J23106 (Promoter2)	Plate1, Well 22A	Chloramphenicol
J23117 (Promoter3)	Plate1, Well 22K	Chloramphenicol
I13504 (GFP)	Plate4, Well 21J	Ampicillin

Transformation and Inoculation:

- The above biobrick parts were revived from the **iGEM 2015 distribution plates** using the iGEM protocol.
- Ultra-competent cells for DH5α strain of *E.coli* were made following the protocol for ultra-competent cell making by iGEM (put link of the page if possible).
- Transformation efficiency was calculated using the **transformation efficiency kit** which was provided by iGEM. The efficiency which was obtained was 4.99*10^7 cfu/ug.
- The parts were transformed and the colonies were obtained when plated on agar+antibiotic.
- The colonies of the biobricks were inoculated in LB with the respective antibiotic and harvested overnight at 37℃
- The plasmid was isolated using Qiagen Mini Kit.
- A preparative gel was run with the single cut restriction enzyme digest to check the plasmid size and concentration.

Cloning:

Restriction Digestion:

(all volumes in uL)	Promoter 1 (J23101)	Promoter 2 (J23106)	Promoter 3 (J23117)	GFP (I13504)	Backbone (pSB1C3,pSB1K3)
DNA	1.1	1.6	1.5	2.2	10
10X NEBuffer2	2.5	2.5	2.5	7.5	2.5
100X BSA	0.5	0.5	0.5	1.5	0.5
EcoRI	0.5	0.5	0.5	-	0.5
SpeI	0.5	0.5	0.5	-	-
XbaI	-	-	-	1.5	-
PstI	-	-	-	1.5	0.5
DpnI	-	-	-	-	0.5
distilled water	14.9	14.5	14.5	45.8	5.5
Total Reaction Volume	**20**	**20**	**20**	**60**	**20**

- Kept at 37°C overnight.
- Heat inactivation of the enzymes at 80℃ for 20 min.

Ligation:

One pot reaction was carried out according to the iGEM Protocol.

(all volumes in µL)	**Ligation Mix**
Plasmid Backbone	2
Promoter Fragment	2
GFP Fragment	2
T4 DNA Ligase	0.5
T4 DNA Ligase Buffer	1
Distilled Water	2.5
Total Reaction Volume	**10**

- Kept at 25℃ for 1 hour
- Heat inactivation of the enzymes at 80℃ for 20 min.
- 2µL of this ligated product was used for transformation using **DH5α** strain of ***E.coli***