Improved blue light repressible promoter

MONDAY, 10/06/2019

Primers arrived in the morning.
We used templet No. 1 which is taken into our iGEM 2019 plasmid box
PCR from 11 am - 1 pm
4 PCR tubes to copy 4 fregments, 11bp spacer fregment 1, 11bp spacer frement 2, 30bp spacer fregment 1 and 30bp spacer fregment 2. Fregemnt 1 length both both are 1.8 kbp, Fregment 2 length for both are 2.3 kbp
After PCR, gel electrophoresis was performed to confirm the fregment lenth which were correct.
We extracted the gel using standard protocol and isolated the fregments.
Gibson Assembly was carried out after we got out fregments. We had two construct of 11bp spacer plasmid and 30bp spacer plasmid.
The Gibson Assembly product were transformed into 10 beta competent cells. The original templet were also transformed into another cell. There are 3 tubes of transformed cells in total. Labelled 0bp, 11bp and 30bp.
150uL of each transformed cells are plated onto three different agar plates with the label corresponding to the transformed cells
Overnight incubation of Agar plates in 37 degree Incubator

Tue, 11/6
All three plates have colonies found on them.
3 colonies are picked from each plate(0bp, 11bp and 30bp), the 30 bp plate had the most red coloration while the 0 bp cannot really see.
Each colony is inoculated into a 50 mL tube. There are 9 tubes in total. 0bp C1 C2 C3, 11bp C1 C2 C3, 30bp C1 C2 C3
All 9 tubes are incubated in the 37 degree incubator

Wed, 12/6
miniprep was performed to all 9 tubes.
500uL of the cells from each tubes were transferred to 1.5 ependoff tube to be kept
1000uL of the cells from each tubes were tranferred to 1.5 ependoff tube in case we want to characterize them in 10 Beta(unlikely)
Plasmid with 0bp 11bp and 30 bp base pairs are extracted from the culture. They are named as 0bp C1, 0bp C2, 0bp C3, 11bp C1, 11bp C2, 11bp C3, 30bp C1, 30bp C2, 30bp C3.
8 uL solutions are taken from each plasmid solution and sent for sequencing, sequencing primer is a forward primer binding the the sequense of DNA right before the bba promoter and before the spacer region. Primers for all plasmid are the same.
The rest are kept in the plasmid box

Thur, 13/6
Sequencing results are back, all 9 results are correct. 1,2,3 align perfectly with 0 bp, 4,5,6 align perfectly with 11bp while 7,8,9 align perfectly with 30bp

Fri, 14/6
Transform the 9 plasmid into 9 MG1655 competent cells and plate them on C plate, leave them on the cupboard and let them grow over weekend for characterization next week.

Mon, 17/6
All night plates with MG1655 had colonies growing. 1 colony from 0bp C1, 1 colony from 11bp C1 and 1 colony from 30bp C1 plate were picked and innoculated into 3 50mL tubes labelled 0bp Mg1655, 11bp MG1655, 30bp MG1655. The plates are sealed with parafilm and stored in 4 degree fridge and the 50mL tubes are left in the incubator in BSL2 lab to grow overnight.

Tue, 18/6
400 uL of each of the overnight incubated cultures were stored in 4degree fridge. Miniprep the overnight incubatored MG1655 cells with 0bp 11bp and 30bp. The plasmid is sent for sequencing just to confirm that the sequence is correct in MG1655, no mutation occur.

Wed, 19/6
Sequencing results are back and the sequences are correct.
Good results!

Thur, 20/6
200uL of each of the 0bp, 11bp and 30bp MG1655 cells are added into 500uL glycerol and shake well then store into the -80 degree freezer. Waiting for characterization.

Tue, 25/6
100uL of the glycerol stock of 0bp 11bp and 30bp MG1655 from the -80 degree freezer were innoculated into 5mL LB+C medium X3. Keep in BSL2 incubator for characterisation on Wednesday.

Wed, 26/6
50uL of overnight culture of 0bp 11bp and 30 bp MG1655 cells are refreshing into 5mL LB+B medium in 3 50mL tubes. Refresh fro 1 and half hours until OD reach about 0.1 to start characterization.
2 of the 12 well plates were used for characterization. The plates layout are the same for both plates.
Plate layout: first column: triplicate of 0bp MG1655, second column: triplicate of 11bp MG1655, third column: triplicate of 30bp MG1655, last column, triplicate of blank LB medium. Picture to be uploaded

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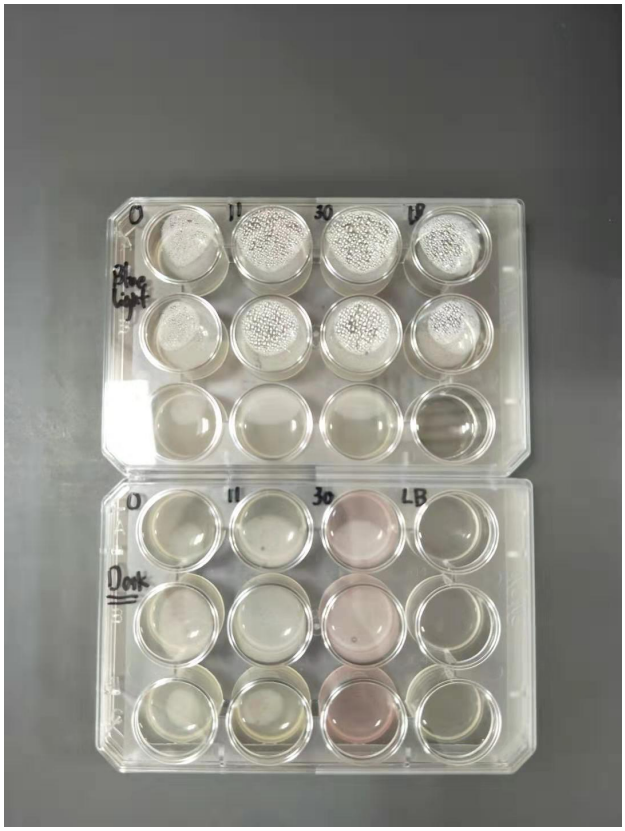

1mL of each cell and medium culture are transferred into each well in the 12 well plates. First reading was taken at 0h time point using H1 synergy microplate reader. The protocol is shaking for 10s, read OD, then read RFP. The results are exported as excel. For the subsequent readings, the protocol is the same throughout and all results are exported in the excel sheet.
After first reading, 1 of the plate is put on the blue light device to be exposed to blue light, while the other plate is covered with a blcak cloth to prevent any exposure to light and therefore repression. Both are incubated in a small shaking incubator. Temperature 37 degree and shaking speed is 125rpm. Hourly reading was performed for the next 5h to collect data.

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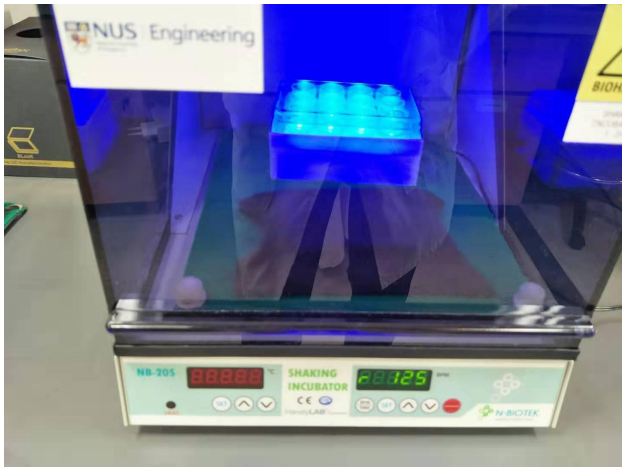

The results are as shown

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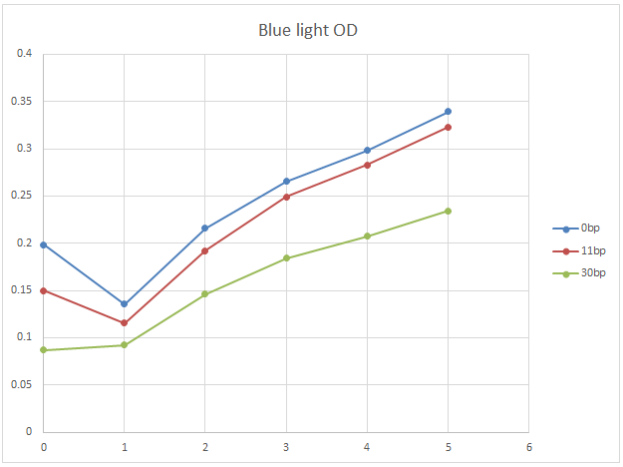

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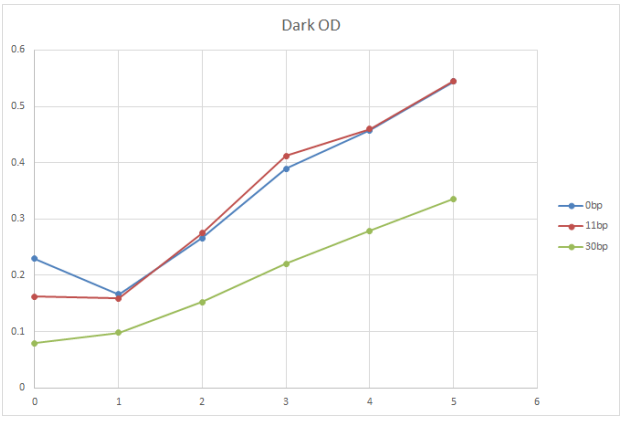

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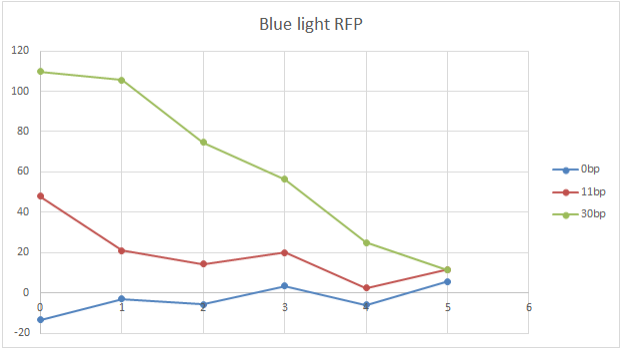

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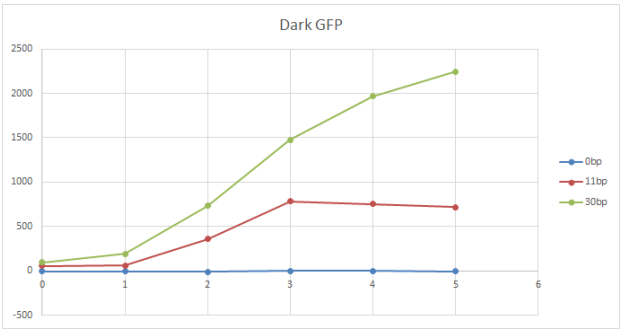

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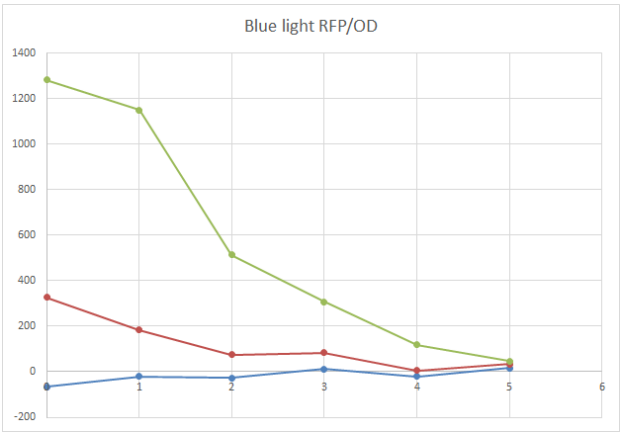

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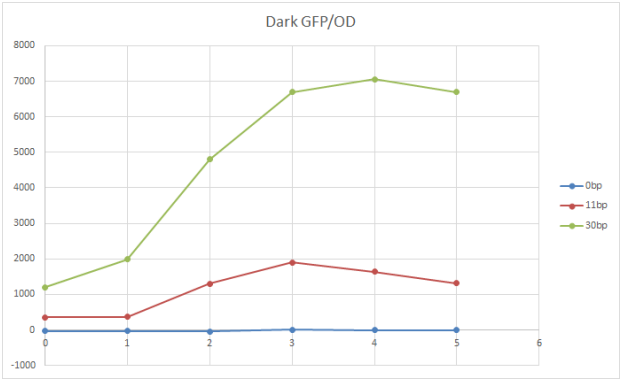

Mon, 1/7

100uL of the glycerol stock of 0bp 11bp and 30bp MG1655 from the -80 degree freezer were innoculated into 5mL LB+C medium X3. Keep in BSL2 incubator for characterisation on Tuesday.

Tue, 2/7

80uL of overnight culture of 0bp 11bp and 30 bp MG1655 cells are refreshing into 8mL LB+B medium in 3 50mL tubes. Refresh fro 1 and half hours until OD reach about 0.1 to start characterization.

Wed, 3/7

Characterization is done again. The procedures are exactly the same as the previous characterization on 26/6. However, more readings are taken up to 8h. This is to ensure a more comprehensive set of results can be collected. Beside, the 50 mL tubes used to refresh the cell culture were wrapped with an aluminium foil to minimize the repression of RFP production before the characterization. This is ensure a higher RFP level at the start of the experiment so that the repression by blue light can be seen more clearly.

The results are presented here.

The different graphs representing OD, RFP and RFP/OD are plotted seperately. The blue light repression is also plotted altogether with the control which is the dark.

IP_2_july_rfp.xlsx

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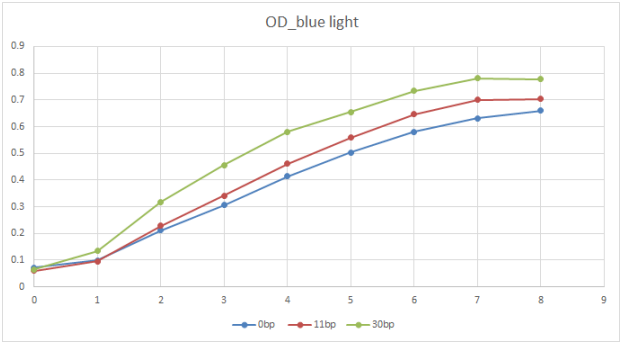

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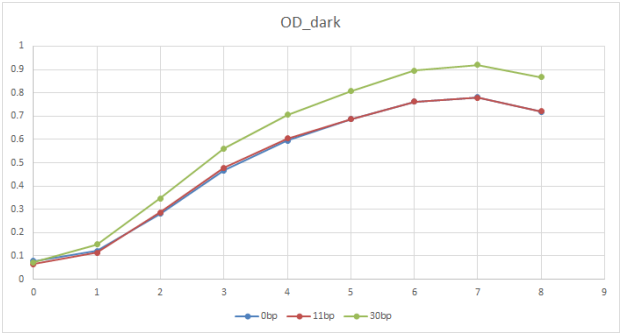

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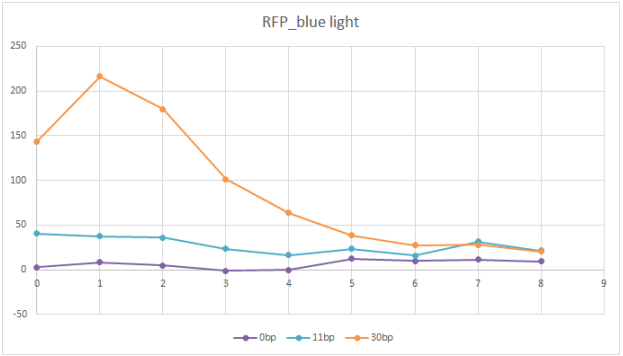

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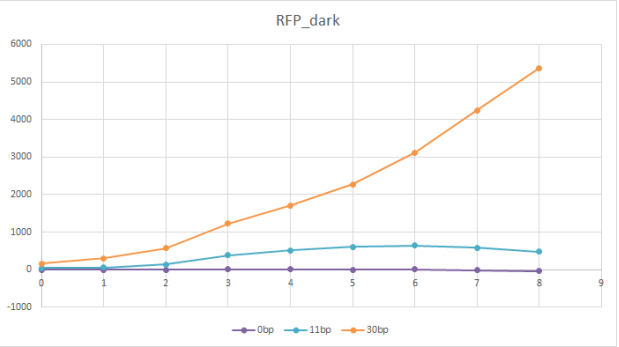

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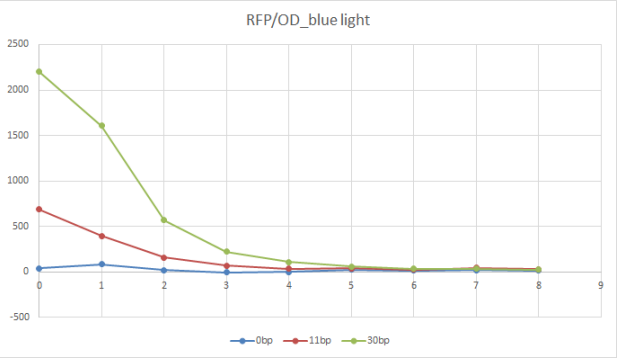

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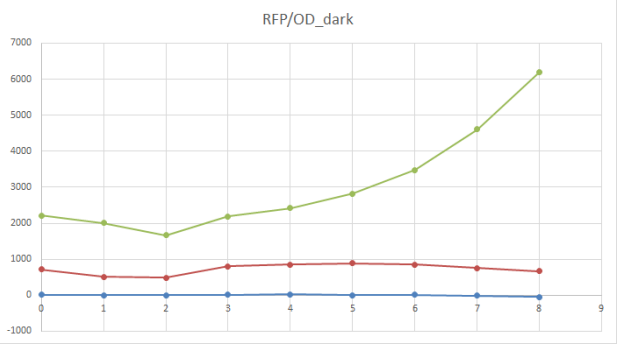

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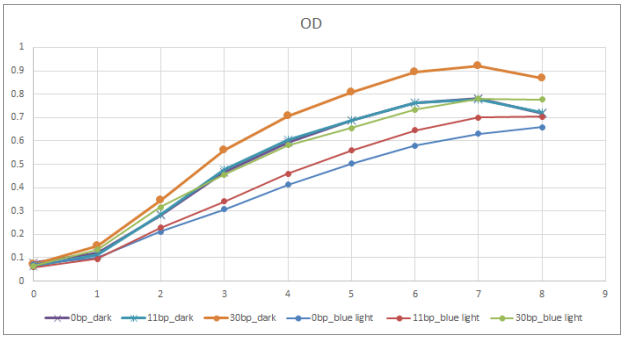

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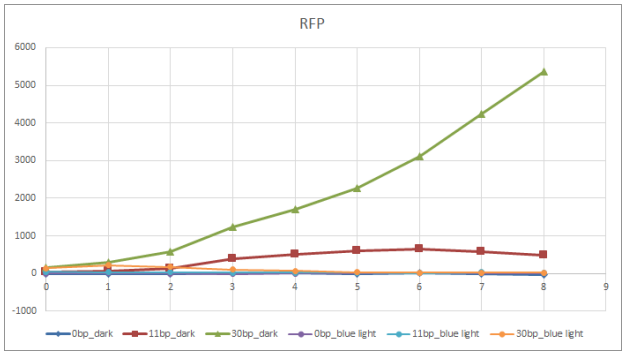

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