**iGEM NOTEBOOK – 2017**

**REGULOGEM**

**FEBRUARY 2017:**

**1st WEEK (1.2.17- 7.2.17):**

Held selection for the team members and gave a brief intro about iGEM and it’s significance.

**2nd  WEEK (8.2.17- 14.2.17):**

Brainstormed various ideas and dropped them due to lack of robustness.

**3rd WEEK (15.2.17- 22.2.17):**

Brainstorming of ideas

Created our CROWD-FUNDING page to raise funds for registration.

**4th WEEK (23.2.17-28.2.17):**

Spread the word about our crowd-funding page through social media.

Approached industries and other personal contacts for funding.

**MARCH 2017:**

**­1st WEEK (1.3.17- 8.3.17):**

Brainstormed more ideas

Fund-raising for registration.

**2nd WEEK (9.3.17- 15.3.17):**

After months of brainstorming ,we finally arrived at our idea “ REGULOGEM”

The proposal was drafted to be sent to Department of Bio-technology(DBT),INDIA for funding

Our proposal was sent for the iBEC competition,an initiative by DBT for funding the Indian iGEM teams.

**3rd WEEK (16.3.17- 22.3.17):**

Visited industries for fund-raising

Got support through our crowd-funding page

**4th WEEK (24.3.17- 31.3.17):**

Fund-raising

Successfully registered for iGEM 2017.

**APRIL 2017:**

**1st WEEK (1.4.17- 7.4.17):**

Chemicals and enzymes were brought for carrying out lab work.

Glass wares, tips and other requirements were sorted out.

**2nd WEEK (8.4.17- 14.4.17):**

Decantation and sterilization was done

The labs were set up for work.

**3rd WEEK – MAY 2nd WEEK:**

No work was done due to exams.

**MAY 2017:**

**3rd WEEK (25.5.17- 31.5.17)**

The team was split into two to carry out Human practices and lab work.

The labs were fumigated and sterilised

The lab team was given training on basic lab techniques

**JUNE 2017:**

**1st WEEK (1.6.17 – 7.6.17)**

The buffers necessary to carry out molecular biology works were prepared.

The Antibiotics stocks were prepared and their activity was checked.

**2nd WEEK (8.6.17 – 14.6.17)**

No lab work

**3rd WEEK (15.6.17 – 22.6.17)**

Prepared the mother culture of E.Coli DH5α.

Optimization of Transformation using the Competent cells prepared using CCMB80 protocol.

Failure of transformation.

We thought that the problem was with buffer.

Optimization of transformation using the competent cells prepared using CaCl2 protocol.

Failure of transformation.Thought that the problem was with the culture

**4th WEEK (25.6.17 – 30.6.17)**

Transformation using the competent cells E.Coli DH5α prepared using ccmb80 protocol and the cells from NEB kit.

Transformation using the cells from NEB kit was successful whereas transformation using the cells from our lab didn’t yield good results.

We confirmed that the problem was with the cells.

**JULY 2017**

**1st WEEK (1.7.17 – 7.7.17)**

Prepared the pure culture of E.Coli DH5α using macconkey agar and then subcultured.

Optimization of transformation using the E.Coli DH5α.

Successfully transformation was optimized.

**2nd WEEK (8.7.17 – 13.7.17)**

PCR amplification of BBa\_J04450 part and No amplification.

The problem was found to be with Primers & Primers were ordered.

Plasmid isolation of RFP from the transformed cells

PCR amplification of the isolated plasmid RFP using the new primers.

No amplification. The problem may be due to enzyme.

PCR amplification of isolated plasmid RFP using master mix & The RFP was amplified.

**3rd WEEK (14.7.17 – 20.7.17)**

Optimization of restriction double digestion at E and P site.

Restricted sample was resolved in gel.

Ligation of the restricted sample with backbone.

Transformation of ligated product.

No growth was observed in the plate containing the cells with ligated product.

Ligation didn’t yield positive result.

**4th WEEK (21.7.17 – 27.7.17)**

PCR amplification of the gblock

No amplification.

But the rfp which was kept as control got amplified.

PCR amplification of gblock ( adaptor with constitutive RBS )

PCR amplification of the gblocks ( pH riboswitch )

Multiple PCR amplication of pH riboswitch

Elution of PCR product

**AUGUST 2017**

**1st WEEK (1.8.17 – 7.8.17):**

Transformation of INTERLAB devices 1,5 and -ve control.

PCR amplplification and gel electrophoresis of gblock pH I and pH II constructs with bio-brick scar and wild scar respectively.

**2nd WEEK ( 8.8.17 – 14.8.17):**

Checking for presence of new gblock samples ( pH I + Adaptor,RNA.T + Adaptor , pH I & II ) ;pH I & II were present.

Isolation of GFP plasmid.

Restriction digestion (double digestion ) of RFP using E & P cut (no result)

**3rd WEEK (15.8.17 – 21.8.17):**

Transformation of interlab devices.

No results were obtained

Transformation of interlab devices

Got positive results. Proceeded for the expression studies.

**4th WEEK (22.8.17 – 28.8.17):**

PCR amplification of pH riboswitch with wild scar, pH riboswitch with biobrick scar ( II time ordered sample).

The gblocks were amplified and multiple PCR was done.

Restriction followed by ligation was performed.

But the sample hasn’t ligated.

We trouble shooted that there was not enough concentration of the insert

**SEPTEMBER 2017**

**1st WEEK ( 1.9.17 – 7.9.17 ):**

Optimization of LBK medium ( modified LB medium – Instead of NaCl, KCl is used ) of following pH : 6.5, 7.0, 7.5, 8.0, 8.5.

Growth curve was studied in LBK medium of different pH (6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 )

The optimum pH of E.Coli in DH5α is found to be 7.5.

No growth was observed in 10 and above.

**2nd WEEK ( 8.9.17 – 14.9.17):**

Restriction digestion of RFP ( for backbone and insert ) and GFP ( for backbone) at E & P site ( one hour incubation ).

RFP was restricted.

**3rd WEEK ( 15.9.17 – 21.9.17 ):**

No work due to assessment test.

**4th WEEK ( 22.9.17 – 28.9.17 ):**

Optimization of transformation using ampicilin.

Blue white screening also performed.

Transformation was good but the efficiency was low.

We witnessed the problem may be with the cells.

The cells were revived.

The blue colonies was sub-cultured into the broth containing ampicillin.

**OCTOBER 2017**

**1st WEEK ( 3.10.17 – 7.10.17 ):**

Transformation using ampicillin was performed again since, the previous transformation hasn’t yield good results (low efficiency).

Transformation was performed again. Got good results with high efficiency.

Restriction of GFP at E & P site & No results.

**2nd WEEK ( 8.10.17 – 14.10.17 ):**

PCR amplification of pH riboswitch with wild scar, pH riboswitch with biobrick scar, Adaptor with RNA Thermometer, Adaptor with pH riboswitch, Adaptor with Constititive RBS, RNA thermometer optimized for Bacillis subtilis.

Multiple PCR & PCR clean up was done.

The multiple PCR products were pooled in and restricted at E & P site.

Simultaneously linearized plasmid backbone ( pSB1C3 ) was also restricted.

The restricted samples are ligated ( incubation 1hour at 16o C ).

Finally, the ligation was successful.

The samples (parts) were arrived from genscript.

The parts were transformed and got fruitful results.

**3rd WEEK ( 15.9.17 – 22.10.17 ):**

The fluorescence expression of the parts were studied.

**25.10.17 -** Parts were shipped to the iGEM headquarters