Month of Recruitment and Meetup: March-April

The recruitment for joining NTU Singapore iGEM team take about 3-4 weeks, starting from 1March to 25March2016. This recruitment was conducted by our Professor Tan Meng How. On 28 March, NTU iGEMers were gathered for a brief introduction of our members and followed by a brainstorming session. Several CRISPR-Cas based projects that are potential to be investigated for a better future application are discussed together with iGEMers and Professor Tan.

We ended up with 3 projects:

1) Evaluation of CRISPR-Cas systems from different bacterial species
2) Improving CRISPR-Cas9 technology for better genome engineering by directed-evolution
3) Truncation of dCas9 by rational design

From1/4-14/4: As some of our teammates are from chemical and biomedical school background while some are from biological sciences background. With different knowledge background on this CRISPR-Cas system, each member is responsible to do a deep research on the related projects and understand comprehensively the principle of CRISPR-Cas.

15/4: Each member was allocated to the projects separately and assigned to different advisors. A google drive and whatsapp group were created for a more convenient discussion.

Month of Training and Practicing: April

15/4-30/4: We were trained for basic technical skills such as cloning, bacterial transformation, miniprep, maintain mammalian cell culture, transfection. In the meantime, we had finished cloning gRNA cloning into plasmid vector and get ready for evaluation projects.

Besides, starting from April, our team were busy in finding funding to support our iGEM projects. Several proposals were written and sent to biotechnology companies. It was the hard time to look for sponsorship as most of the company did not response their interest to our project. Finally, Agency for Science technology and Research (A*STAR), NTU School of Chemical and Biomedical Engineering (SCBE) and lastly School of Biological Science (SBS) were willingly support us for this iGEM competition.

Month of Experiments: May- October

May:

The 3 projects were conducted concurrently under guidance of different advisors throughout this iGEM preparation periods.

For Evaluation project: Part of characterization of Cas9/Cpf1 efficiency from different species based on different promoters had been done by T7E assays. For T7E assays, it was quite troublematic by just adding 0.5ul of T7 endonuclease into reactions, especially when a large number of reactions are conducted simultaneously.

For Evolution project: A random mutagenesis was conducted followed by mutagenesis screen. Selected mutant variants were sent for sequencing to confirm mutation sites.

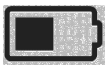
For dCas9 truncation project: Reporter activation using wild type dCas9 was firstly to be tested. Conducted structural study to identify potential sites of dCas9 to be truncated using PyMOL software. By Gibscon assembly, plasmid constructs carrying dCas9 with full truncated nuclease domains such as HNH, RuvCII and RuvCIII were generated. Tested reporter activation with nuclease-truncated dCas9 on HEK293FT cell. FACS analysis.

 *June:*

For Evaluation project: Produce second biological replicate of results about characterization of Cas9/Cpf1 efficiency from different species based on different promoters had been done by T7E assays. Additionally, characterization of gRNA length use for genome targeting was done as well.

For Evolution project: After sequence confirm, 8 rounds of directed evolution were done.

For dCas9 truncation project: Pinpointing truncated sites for RuvCIII. Tested reporter activation with RuvCIII-truncated dCas9 on HEK293FT cell. FACS analysis.

 *July:*

For Evaluation project: Produce third biological replicate of results about characterization of Cas9/Cpf1 efficiency from different species based on different promoters using T7E assays. Continue to characterize the remaining gRNA length for better genome targeting.

For Evolution project: Repeat competition assays to look for repeated appeared mutants.

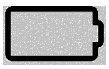
For dCas9 truncation project: Truncation of REC1 domain of dCas9. Tested reporter activation with RECI-truncated dCas9 on HEK293FT cell. FACS analysis.

 *August:*

For Evaluation project: As the experiments to obtain third replicate of results failed, steps stated above for characterization of Cas9/Cpf1 efficiency from different species based on different promoters were repeated.

For Evolution project: To characterize the efficiency of SpCas9 mutant by cloning gRNA into plasmid.

For dCas9 truncation project: Truncation of PI domain of dCas9. Tested reporter activation with PI-truncated dCas9 on HEK293FT cell. FACS analysis.

 *September:*

For Evaluation project: To produce second biological replicate of results about characterization of gRNA length for better genome targeting.

For Evolution project: Characterize cutting efficiency of SpCas9 variants on mammalian cells using T7E assays. Continue with construction of targeting plasmid.

For dCas9 truncation project: Continue to truncate different parts of PI domain of dCas9. Tested reporter activation with PI-truncated dCas9 on HEK293FT cell. FACS analysis.

Additionally, some of our members had started to work on the iGEM Wiki. This is quite challenging task to some of us as we did not really good in programming. However, it was indeed a good chance for us to learn things beside synthetic biology.

 *October:*

We started for part submission works! :) In the meantime, projects were continued.

For Evaluation project: To produce third biological replicate of results about characterization of gRNA length.

For Evolution project: Characterize cutting efficiency of SpCas9 variants on mammalian cells using T7E assays. FACS preparation, genomic extraction, PCR amplification of target sites , anneal PCR products and lastly T7E assay were involved.

For dCas9 truncation project: Combine all potential to be truncated domains of dCas9 as shown from the previous results. Further demonstration of truncated dCas9 by activating ADAR gene in H1 ES cells.