Practical guide for starting a Synthetic Biology club

BIOSIN UFMG

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PRACTICAL GUIDE INTRODUCTION

This practical guide was developed by the Club of Synthetic Biology and Genetic Engineering at UFMG, aiming to show the step-by-step of the creation and development of a club. In particular, we hope that this guide can serve as an excellent tool to assist in the creation of a synthetic biology club.

The guide contains relevant information such as selection of members, development of knowledge and organization of topics.

We emphasize that each group has its own time, it is not mandatory to follow the chronological order of the information mentioned here, except for Module 1 - Leveling.

APPLICATION AND SELECTION

When selecting for the club, outline the values you want for new club members.

A tip that can be followed is to disregard the academic level of the selected member, so you can create a more diverse group, ranging from high school students to doctors. Prioritize dedication, demonstration of commitment and interest in the area. With that in mind everything should be done depending on the profile of the club soon to be created. We emphasize that you must outline the profile of candidates that best suits your study, discussion and project group.

To facilitate the selection process, it is possible to find in this guide a form with questions for candidates about name, degree level, if they are available to attend meetings on the proposed day and time, qualities and flaws, if they have experience with areas co-related to synthetic biology. Do not be alarmed if few or no candidates have experience specifically with synthetic biology. As it is an area of recent development, most of those interested will have no practice in the area.

Module 1

LEVELING

Module 1 - Leveling

This module aims to level the knowledge of the members, considering the different experience of each one of them. In this module they should learn about basic concepts of molecular biology, with an emphasis on DNA replication and the central dogma of molecular biology, transcription and translation.

We recommend that a master, doctor or professor with experience in molecular biology conduct this initial exploration. In addition, it is important to maintain a multilateral dynamic, talking to members to ensure understanding. Unlike an expository class, here it is a space for conversation, if the members of the club did not understand any aspect it is important to bring up the same topic in the next meeting, until everybody is on the same page.

If necessary, we recommend the use of images and videos in order to clarify the explanations and facilitate understanding.

Initial Meeting

The first club meeting should be used to introduce the team and members. After this first contact, it is interesting to contextualize what synthetic biology is all about. In addition to talking a little about the history of synthetic biology, it is important to contextualize the information, exposing projects and works in the area, always paying attention to explain details and technical terms that may be used.

We especially recommend the articles by Meng et al. (2020) and Cameron at al. (2014) to contextualize the information to be passed on.

Suggestion for presentation by members, questions that can be asked:

"How do you want to be called?"

"What is your area of study?"

"Why do you want to know more about synthetic biology?"

Recommended material:

MENG, Fankang; ELLIS, Tom. The second decade of synthetic biology: 2010:2020. **Nature Communications**, [S.L.], v. 11, n. 1, 14 out. 2020. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/s41467-020-19092-2.

CAMERON, D. Ewen; BASHOR, Caleb J.; COLLINS, James J.. A brief history of synthetic biology. **Nature Reviews Microbiology**, [S.L.], v. 12, n. 5, p. 381-390, 1 abr. 2014. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nrmicro3239.

DNA replication

This will be the first meeting in which technical aspects will be addressed, so it is interesting to contextualize what DNA is, where it is located and what its functions are.

From then on, DNA replication and repair mechanisms and their application in Synthetic Biology must be explained and elucidated. The work of van Nies et al. (2018) is a good example of how understanding this mechanism led to the development of parts that reproduce the work of cellular machinery, but in a cell-free context.

Recommended material:

SCHRANK, Irene Silveira. Replicação do DNA. In: ZAHA, Arnaldo; FERREIRA, Henrique Bunselmeyer; PASSAGLIA, Luciane M. P. **Biologia Molecular Básica**. 5. ed. São Paulo: Artmed, 2014. p. 111-132.

ALBERTS, Bruce *et al*. Replicação, reparo e recombinação do DNA. In: ALBERTS, Bruce *et al*. **Biologia Molecular da Célula**. 6. ed. São Paulo: Artmed, 2017. p. 237-298.

VAN NIES, Pauline; WESTERLAKEN, Ilja; BLANKEN, Duco; SALAS, Margarita; MENCÍA, Mario; DANELON, Christophe. Self-replication of DNA by its encoded proteins in liposome-based synthetic cells. **Nature Communications**, v. 9, n. 1, 20 abr. 2018. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/s41467-018-03926-1.

Transcription

The third meeting should be held to elucidate the mechanisms of transcription and post-transcriptional modifications, for both prokaryotes and eukaryotes. It is necessary to emphasize some parts, such as promoters, sigma factor and transcription factors that are important components for understanding and are relevant to the development and applications of Synthetic Biology.

In the Engstrom and Pfleger, 2017 review article, we can see several applications with transcription engineering. Among them, the use of promoters and engineered terminators for transcription modulation as well as the creation of Boolean switches responsive to various signals from cellular environment. The study model was based on E.coli, but there are examples of the use of these tools in other organisms.

Recommended material:

SCHRANK, Augusto. Transcrição. In: ZAHA, Arnaldo; FERREIRA, Henrique Bunselmeyer; PASSAGLIA, Luciane M. P. **Biologia Molecular Básica**. 5. ed. São Paulo: Artmed, 2014. p. 205-232.

ALBERTS, Bruce *et al*. Como as células leem o genoma: do DNA à proteína. In: ALBERTS, Bruce *et al*. **Biologia Molecular da Célula**. 6. ed. São Paulo: Artmed, 2017. p. 299-268.

ENGSTROM, Michael D.; PFLEGER, Brian F.. Transcription control engineering and applications in synthetic biology. **Synthetic And Systems Biotechnology**, v. 2, n. 3, p. 176-191, set. 2017. Elsevier BV. http://dx.doi.org/10.1016/j.synbio.2017.09.003.

Translation

In this fourth meeting, the mechanisms of translation and their post-translational modifications must be clarified. It is important to paying attention to the differences in the mechanisms of prokaryotes and eukaryotes. In order to illustrate the concepts, we can mention the article by Jeffery M et al, 2020 which is about the expansion of the genetic code generating a system that recognizes unconventional amino acids.

Recommended material:

SCHRANK, Irene; VAINSTEIN, Marilene Henning. Transcrição. In: ZAHA, Arnaldo; FERREIRA, Henrique Bunselmeyer; PASSAGLIA, Luciane M. P. **Biologia Molecular Básica**. 5. ed. São Paulo: Artmed, 2014. p. 205-232.

ALBERTS, Bruce *et al*. Como as células leem o genoma: do DNA à proteína. In: ALBERTS, Bruce *et al*. **Biologia Molecular da Célula**. 6. ed. São Paulo: Artmed, 2017. p. 299-268.

THARP, Jeffery M.; KRAHN, Natalie; VARSHNEY, Umesh; SÖLL, Dieter. Hijacking Translation Initiation for Synthetic Biology. **Chembiochem**, [S.L.], v. 21, n. 10, p. 1387-1396, 2 mar. 2020. Wiley. http://dx.doi.org/10.1002/cbic.202000017.

Module 2

INTRODUCTION

Module 2 - Introduction

In module 2, the participation of members becomes more essential, being interspersed with lectures. Club members should be divided among themselves, to research and answer questions that appeared during the meetings, we suggest some subjects in the Discussion topic. The questions aim to elucidate parts of the essential processes for knowledge consolidation.

The lectures must follow the same dynamics as the previous module. Emphasizing that presentations of basic topics should preferably be given by a person with experience. Considering that the club has members from different areas, they themselves can be the speakers.

Logic Gates and Bases of Genetic Circuits

Meeting goal: Present the concepts of Biological Parts and in a playful way to correlate them with the concept of Parts in Engineering. We recommend the presentation of the concepts of Boolean operators, toggle switch, repressilators and self-regulatory circuits. These concepts make it possible to explain genetic circuits to members.

Recommended material:

GARDNER, Timothy S.; CANTOR, Charles R.; COLLINS, James J.. Construction of a genetic toggle switch in Escherichia coli. **Nature**, [S.L.], v. 403, n. 6767, p. 339-342, jan. 2000. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/35002131.

ELOWITZ, Michael B.; LEIBLER, Stanislas. A synthetic oscillatory network of transcriptional regulators. **Nature**, [S.L.], v. 403, n. 6767, p. 335-338, jan. 2000. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/35002125.

Molecular Biology techniques applied to Synthetic Biology

Meeting goal: Obtain an introductory understanding of the biomolecular tools and techniques used in laboratories, such as gel electrophoresis, transformation techniques, PCR and cloning, with the objective of promoting an applied thinking of these techniques to Synthetic Biology.

Recommended material:

ALBERTS, Bruce *et al*. Como as células leem o genoma: do DNA à proteína. In: ALBERTS, Bruce *et al*. **Biologia Molecular da Célula**. 6. ed. São Paulo: Artmed, 2017. p. 299-268.p. 501-578.

Computational modeling

Meeting goal: Show the most relevant bioinformatics tools and databases for analysis and comparison of DNA sequencing as well as promoting an introductory notion of the use of these tools and interpretation of the results obtained. Contextualize the tools within synthetic biology. Some of the tools used to contextualize the objectives of this class are UniProt, RCSB PDB - Protein Data Bank, iGem Parts, BLAST and ClustalW

Recommended material:

Andreas D. Baxevanis et al, B. F. Francis Ouellette et al. **Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins.** 4. ed. Wiley, 2020. p. 187-212.

Discussions

In this part of the module, the members of the club began to have a more active approach for the development of knowledge, so they separated themselves into groups so that they could answer questions that were raised in previous lectures.

Bear in mind that these questions are just a suggestion, other doubts can be raised, we suggest the creation of groups to clarify doubts.

A. A. The alternative splicing phenomenon allows the production of several proteins from a single gene. Discuss the importance of this process in the generation of antibodies by B cells. Describe another example of alternative splicing with medical importance.
B. Point out an example of iGEM project in which constructions with strong and weak promoters have been compared, and a project that combined the use of constitutive and inducible promoters. Discuss the results obtained and the choice of promoters.
C. Explain and cite studies on the use of the tryptophan operon, compare its mechanism with the lac operon.
D. How many types of tRNA are described in prokaryotes and how many in eukaryotes? What is the reason for this difference? How many types of Amino acyl tRNA synthetases are there? How to produce a synthetic tRNA? Name a project that uses synthetic tRNA.
E. What is the relationship between the ribosome structure and its function? Differences between prokaryotes and eukaryotes? Interactions with other proteins? How difficult is it to make a synthetic ribosome?
F. Name two literature projects that involve artificial proteins with translation modification strategies.

Discussion: Alternative splicing

Meeting goal: Discuss the importance of the alternative splicing process in the generation of antibodies by B cells. In addition, exemplify the use of this process in other medical activities in order to contextualize its application.

Recommended material:

SCHAUB, Annalisa; GLASMACHER, Elke. Splicing in immune cells—mechanistic insights and emerging topics. **International Immunology**, [S.L.], v. 29, n. 4, p. 173-181, 1 abr. 2017. Oxford University Press (OUP). http://dx.doi.org/10.1093/intimm/dxx026

RIGHETTO, Germanna Lima. **SF2/ASF e SRPK2 : relação entre a maquinaria de splicing alternativo e o desenvolvimento da leucemia**. 2013. 81 f. Dissertação (Mestrado) - Curso de Genética e Biologia Molecular, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, 2013. (*Material in Portuguese*)

Discussion: RNA polymerase sigma subunit

Meeting goal: Explain how the sigma subunit recognition of DNA occurs as well as the activation of transcription. Address the difference of this process in prokaryotic and eukaryotic organisms.

Recommended material:

YELLESWARAPU, Maaruthy; LINDEN, Ardjan J. van Der; VAN SLUIJS, Bob; PIETERS, Pascal A.; DUBUC, Emilien; GREEF, Tom F. A. de; HUCK, Wilhelm T. S.. Sigma Factor-Mediated Tuning of Bacterial Cell-Free Synthetic Genetic Oscillators. **Acs Synthetic Biology**, v. 7, n. 12, p. 2879-2887, 8 nov. 2018. American Chemical Society (ACS). http://dx.doi.org/10.1021/acssynbio.8b00300.

Discussion: iGEM projects discussion focusing on promoters choice

Meeting goal: Provide a discussion on how, in practice, the choice of strong and weak promoters works, as well as the combined use of constitutive and inducible promoters, based on the results found in the projects discussed.

Recommended material:

Team Northwestern University iGEM 2013. NU-TRALIZE! :

http://2013.igem.org/Team:Northwestern

Team SCAU-China iGEM 2018. Cupid Project:

https://2018.igem.org/Team:SCAU-China.

Discussion: Tryptophan operon usage and mechanism comparison to Lac operon

Meeting goal: Understand how the use of the tryptophan operon works as well as its effective mechanisms. In addition, make a comparison with Lac operon, due to its historical and research importance.

Recommended material:

Khan Academy. Gene regulation in bacterias:

https://pt.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-bacteria/a/overview-gene-regulation-in-bacteria.

BJERRE, Karin; CANTOR, Mette D.; NØRGAARD, Jan V.; POULSEN, Hanne D.; BLAABJERG, Karoline; CANIBE, Nuria; JENSEN, Bent B.; STUER-LAURIDSEN, Birgitte; NIELSEN, Bea; DERKX, Patrick M. F.. Development of Bacillus subtilis mutants to produce tryptophan in pigs. **Biotechnology Letters**, [S.L.], v. 39, n. 2, p. 289-295, 3 nov. 2016. Springer Science and Business Media LLC. http://dx.doi.org/10.1007/s10529-016-2245-6.

Team UCopenhagen iGEM 2017. Incell CPH:

http://2017.igem.org/Team:UCopenhagen/Project

Discussion: tRNA in prokariotes and eukariotes, how many, what are the differences, how to manufacture them

Meeting goal: Discuss what the different types of tRNAs are described for prokaryotes and eukaryotes and what makes them different. Address the types of Amino acyl tRNA synthetases that exist in nature as well as the production of this type of RNA in a synthetic manner.

Recommended material:

Team: Austin-Texas. Project: Expanded Genetic Code Measurement Kit:

http://2014.igem.org/Team:Austin_Texas

The Editors of Encyclopaedia Britannica. Transfer RNA. Encyclopædia Britannica. January 29, 2014.

RÉDEI, George P. Transfer RNA (tRNA). **Encyclopedia Of Genetics, Genomics, Proteomics And Informatics**, p. 2002-2004, 2008. Springer Netherlands. http://dx.doi.org/10.1007/978-1-4020-6754-9_17259.

Discussion: Relationship between ribosomes structure and function, as well as their interaction with other proteins.

Meeting goal: Establish the relationship between function and structure of the ribosome, highlighting the differences between prokaryotes and eukaryotes. In addition, to address the interactions with other proteins and the difficulty of making a synthetic ribosome.

Recommended material:

DEUSSER, Ellen. Heterogeneity of ribosomal populations in Escherichia coli cells grown in different media. **Molecular And General Genetics Mgg**, [S.L.], v. 119, n. 3, p. 249-258, set. 1972. Springer Science and Business Media LLC. http://dx.doi.org/10.1007/bf00333862.

DEUSSER, Ellen; WITTMANN, Heinz-Günter. Biological Sciences: ribosomal proteins. **Nature**, [S.L.], v. 238, n. 5362, p. 269-270, ago. 1972. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/238269a0.

ELLMAN, J.; MENDEL, D; SCHULTZ, P. Site-specific incorporation of novel backbone structures into proteins. **Science**, v. 255, n. 5041, p. 197-200, 10 jan. 1992. American Association for the Advancement of Science (AAAS). http://dx.doi.org/10.1126/science.1553546.

Discussion: Projects about artificial proteins with strategies to change translation.

Meeting goal: Discuss the use of artificial proteins as a strategy to modify the translation process, promoting greater robustness in the control and translation processes of the cellular environment.

Recommended material:

BARBER, Karl W; RINEHART, Jesse. The ABCs of PTMs. **Nature Chemical Biology**, v. 14, n. 3, p. 188-192, 14 fev. 2018. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/nchembio.2572.

LAJOIE, M.J. et al. Genomically Recoded Organisms Expand Biological Functions. **Science**, v. 342, n. 6156, p. 357-360, 18 oct. 2013. http://dx.doi.org/10.1126/science.1241459

Module 3

BASIC

Módulo 3 – Básico

The third module aims to start the theoretical-practical application of the knowledge assimilated by the members through the previous modules. At every meeting of this module, topics with potential to be further discussed are shown and lightly explained, depending on the interest of the members it can be better studied. Such topics may, in the future, become a synthetic biology project developed by the club.

The structure of the meetings is more decentralized and dynamic, requiring more active participation by members. The topics discussed individually at each meeting are further developed by members through studies of related articles. Thus, it allows a more complete view when exploring different angles of a given theme. .

In this module, we should also explore basic bioinformatics concepts in order to remedy a practical activity that respects social distance due to the pandemic. It is important to propose sequence alignment, identification and clustering exercises using online and open software. It is also possible to invite members of iGEM teams to share their experience in project development and how the competition has impacted their academic career.

After the end of module three, a specific group can be created to develop synthetic biology projects or even a team for iGEM.

Suggested topics:

1 - CRISPR

Discuss the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) as a mechanism for gene editing. Discussing its operation, advantages and disadvantages.

2 - Artemisinin

Artemisinin is an antimalarial of high clinical and pharmacological importance, being natural to the flower of the *Artemisia vulgaris* plant. Its scaling process requires highly relevant chemical and biological industrial techniques and processes.

3 - Synthetic biology - ethics and media

Synthetic biology promotes socio-political impacts of great relevance, spreading across domains of diverse thought and knowledge, such as bioethics, philosophy, economics, among others. Tracing a discussion that understands all those relationships is a valid way of promoting a more integrated and interdisciplinary knowledge about synthetic biology.

4 - Synthetic biology in industry

Synthetic biology is an approach with high potential in the industry, promoting methods of high commercial and eco-sustainable interest. The members could search for applications already in use and propose innovations.

6 - Cleaning hormones in the water treatment plant

Current sewage treatment methods do not contemplate, at least in an efficient way, the elimination of hormones, which in turn can impact the entire ecological chain and ecosystems diversity. Synthetic biology is a tool for the development of alternative methods that address this problem.

7 - Improving inaccurate diagnoses

There are several diseases of high clinical interest that are underreported or late diagnosed. One reason for this is the inaccurate diagnoses, either due to the inherent limitation of the technique or the nature of the disease. Synthetic biology is able to act in both front with different approach.

Application Form - Synthetic Biology Club

Section 1

Name:

Phone:

Email:

Are you available to attend meetings on the date and time of the club reunion?

- ○ YES
- ○ NO

Education level:

- ○ High School
- ○ Undergraduate
- ○ Master
- ○ Doctor
- ○ Other

If coursing:

What is the name of your course:

How far into it are you? semester

Application Form - Synthetic Biology Club

Section 2

Tell us about you!

In the selection process the student's motivation will count more than their experience. So don't be alarmed, we just want to get to know you better!

How did you hear about the Synthetic Biology Club?

Have you ever participated in an undergrad or high school scientific research? If so, what was your line of research? Tell us more about your experience.

Do you have any experience with Molecular Biology, Biotechnology or Synthetic Biology? Tell us about your contact with these areas.

Tell us a little more about your skills, qualities, flaws and most important of all what are your motivations that influence you to participate in the Synthetic Biology Club.