**Optimizing an *in vivo* Mutagenesis Method in *S. cerevisiae* (Master thesis, 6 months)**

**Background and Goal**

We recently developed a platform in yeast (Evolverator) that can perform *in vivo* evolution of binding interactions and will expand this platform to evolve peptides and proteins that bind G-protein coupled receptors (GPCRs). These receptors are important drug targets, and this platform will aim to produce agonists and antagonists for these receptors, with the eventual goal of contributing to drug discovery.

One of the key components of the platform is targeted *in vivo* mutagenesis of the candidate binders. The more efficient this process, the more diversified the library of binders we can screen. In the first iteration of this platform, we used the MutaT7 system[1], [2] together with an adenine and a cytosine base editor. With this system, the DNA polymerase of phage T7 is fused to the base editors. We then place a T7 promoter in front of the gene we want to target. As the gene is transcribed by the T7 polymerase, the base editors introduce mutations. While this system is quite efficient, recent publications have shown that both the type of mutations and its efficiency can be further improved[3].

The challenge is to find the most efficient set up for our specific context. There are many factors to be tested, including the placement and number of T7 promoters, new and improved base editors, as well as additional proteins that have been shown to increase mutation efficiency. The goal of this master project will be to optimize the MutaT7 system for the context of the Evolverator, with which we will first evolve ligands for the mating pathway receptor STE2 as a proof of concept. You’ll be provided with direction and initial ideas, but you will be free (and expected) to bring your own ideas as you gain experience with the method.

**Tasks**

1. Review the literature to create an initial plan of what types of improvements to test and how.
2. *Implementation in the lab*.
	1. Clone all necessary constructs and integrate them into yeast for testing.
	2. Design experiments to determine which improvements are worth pursuing.
	3. Perform fluctuation assays to quantify mutation rates and analyze data in R.
	4. Depending on initial data, more literature review might be required to identify different ways of increasing mutation rates.
3. Once individual improvements that increase mutation rates are identified, you will test whether they are compatible and can work synergistically.
4. *Optional.* There are other published mutagenesis systems with completely different mutagenesis methods. Depending on need and interest, you can implement some of these in our strains and quantify their efficiency.
5. *Reporting.* You will be required to summarize your results in a written report and an oral presentation.

**Skills you will acquire**

You’ll learn how to review literature, and more importantly, how to assess the quality of published data and reimplement published methods yourself. You’ll learn how to design efficient experiments, how to troubleshoot these and how to critically analyze your own data.

On the technical side, you’ll be proficient with yeast handling, yeast transformations, fluctuation assays, various molecular cloning methods, Sanger sequencing and potentially Next-Generation Sequencing by the end of the project. Depending on interest, you can also gain experience in flow cytometry methods. You will also learn how to analyze and present data using R.

**Requirements**

Motivation and a willingness to learn are more important than your background. If you don’t fit the formal requirements below, but you find the project interesting, you are still encouraged to apply.

1. Previous experience in a wet lab through lab courses or short projects. Ideally already familiar with basic methods such as PCR, gel electrophoresis, cell plating etc.
2. Basic knowledge in (synthetic) biology.
3. Any coding experience is a plus but not necessary.

**Supervision**

If interested, send an email to Dr. Asli Azizoglu (asliazizo@hotmail.com ) and Prof. Dr. Jörg Stelling (joerg.stelling@bsse.ethz.ch ).

**References**

[1] C. L. Moore, L. J. Papa, and M. D. Shoulders, “A Processive Protein Chimera Introduces Mutations across Defined DNA Regions in Vivo,” *J Am Chem Soc*, vol. 140, no. 37, 2018, doi: 10.1021/jacs.8b04001.

[2] A. Cravens, O. K. Jamil, D. Kong, J. T. Sockolosky, and C. D. Smolke, “Polymerase-guided base editing enables in vivo mutagenesis and rapid protein engineering,” *Nat Commun*, vol. 12, no. 1, 2021, doi: 10.1038/s41467-021-21876-z.

[3] Z. Huang, X. Chen, D. Liu, Y. Cui, B. Li, and Y. Yuan, “Enhanced single‐base mutation diversity by the combination of cytidine deaminase with DNA‐repairing enzymes in yeast,” *Biotechnol J*, Aug. 2023, doi: 10.1002/biot.202300137.