

# Internship Proposal

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## **Project Title:**

Determining the impact of nucleotide alterations in the non-coding genome using CRISPR/Cas9

## **Level:**

Master Student

## **Project Summary:**

Many diseases, like type 2 diabetes (T2D), are impacted by genomic alterations. Small nucleotide alterations in sequences with transcriptional enhancer activity have been correlated with impaired function of the endocrine pancreas, contributing to T2D. As consequence of nucleotide alterations in enhancers, genes are mis-expressed, impacting cell function. However, it is poorly understood how specific nucleotide alterations in enhancer sequences impact transcription and gene expression networks.

## **Objectives:**

This project aims to generate a dataset of nucleotide alterations in regulatory genomic elements to create machine learning models that predict and explain enhancer activity.

## **Work to be developed by the student:**

### **Methodologies:**

The student will work with mammalian cells, in vitro, and genome-editing tools to target regulatory regions and assess the impact of nucleotide alterations in gene expression and cell function. The resulting data will be used in bioinformatic analysis (participation of the student will depend on his/her previous skills and progression of the project). The student will work closely with PhD students and Postdocs in the lab, in an ongoing project.

-Cell culture

-Cell transfection and infection

-Enhancer reporter assays

-Molecular cloning

-Other molecular biology techniques, such as DNA and RNA extraction

- Design and use of CRISPR/Cas9 tools, including prime editing
- Gene expression analysis, including qPCR
- Preparation of libraries for next-generation sequencing (NGS)
- May include processing of NGS data



Expected results:

- Validation of the activity of putative enhancer sequences.
- Generation of cell populations with edited non-coding regulatory elements.
- Generation of NGS libraries and NGS datasets for machine learning models.
- Description of the role of single-nucleotide alterations in non-coding elements in gene expression, and their impact on gene expression.

### References:

Relevant publications:

F.J. Ferreira, M. Galhardo, J. Nogueira, J. Teixeira, E. Logarinho\*, J. Bessa\*. FOXM1 expression reverts aging chromatin profiles through repression of the senescence-associated pioneer factor AP-1. *Nature Communications* 16, 2931 (2025). \*Corresponding authors.

DOI: <https://doi.org/10.1038/s41467-025-57503-4>

R. Bordeira-Carriço, J. Teixeira, M. Duque, M. Galhardo, D. Ribeiro, R. Dominguez-Acemel, P. N. Firbas, J. J. Tena, A. Eufrazio, J. Marques, F.J. Ferreira, T. Freitas, F. Carneiro, J. L. Gómez-Skarmeta, J. Bessa. Multidimensional chromatin profiling of zebrafish pancreas to uncover and investigate disease-relevant enhancers. *Nature Communications* 13 1 (2022). DOI: <http://dx.doi.org/10.1038/s41467-022-29551-7>

A. Eufrásio, C. Perrod, F.J. Ferreira, M. Duque, M. Galhardo, J. Bessa. In Vivo Reporter Assays Uncover Changes in Enhancer Activity Caused by Type 2 Diabetes-Associated Single Nucleotide Polymorphisms. *Diabetes*, Dec; 69(12): 2794-2805 (2020). DOI: <https://doi.org/10.2337/db19-1049>