Universitat Pompeu Fabra Barcelona | Master in Bioinformatics for Health Sciences

Master project 2024-2025

Personal Information

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Project

Computational systems biology

Project Title:

Understanding splicing using deep mutational scanning

Keywords:

Alternative splicing, hightroughput experiments, mutational effects, Illumina sequencing

Summary:

The regulation of gene expression in eukaryotes relies on intron excision and alternative splicing, which contributes to proteome diversity, cell differentiation, and organismal homeostasis. However, understanding the splicing regulatory code and accurately predicting the effects of splicing-altering variants remain challenging. Approximately 30% of Mendelian disease-causing variants affect splicing, highlighting the importance of improving splicing variant prediction in the clinical genetic field. Furthermore, antisense oligonucleotides (AONs) have emerged as a promising therapeutic strategy for therapeutically modifying splicing, but the identification of targetable regions remains a challenge. To tackle these challenges, we are constructing reference atlases to comprehensively assess the impact of variants on splicing in multiple human exons. We quantify exon inclusion or skipping in cell lines to examine the effects. Our objective is to mutagenize 1000 exons associated with Mendelian diseases and cancer. The student will be involved in both the experimental and computational sides of the project. He/She will learn how to perform the experiment to assess the effect of thousands of variants in parallel, in particular, how to design and clone the variant library, work in a sterile cell culture environment (cell line maintenance and transfection), perform RNA extraction and reverse transcription, prepare the libraries for Illumina sequencing (primer design, qPCR, PCR purification protocols). Furthermore, he/she will learn how to process the Illumina sequencing data with a tool developed in-house (ref 4), quantify the effect of splicing-altering variants, analyse the data combining it with available databases of mutations (e.g., Clinvar, GnomAD), splicing regulatory elements and RNA binding protein, and AON (e.g., eSkip-Finder) to understand how mutations can alter splicing and improve our knowledge about how the cis-regulatory elements of splicing work. The insights gained from our data will shed light on the mechanisms by which mutations alter splicing and help us to identify regions that are sensitive to AONs.

References:

1. Baeza-Centurion et al. “Mutations primarily alter the inclusion of alternatively spliced exons” - eLife 2020 2. Baeza-Centurion et al. “Combinatorial Genetics Reveals a Scaling Law for the Effects of Mutations on Splicing” - Cell 2019 3. Julien et al. “The complete local genotype-phenotype landscape for the alternative splicing of a human exon” - Nature communications 2016 4. Faure AJ et al. “DiMSum: an error model and pipeline for analyzing deep mutational scanning data and diagnosing common experimental pathologies” - Genome Biology 2020

Expected skills:

- Keenness to learn high-throughput methods, in the lab and with respect to data analysis - Basic skills in molecular biology and cell culture - a programing language (R, bash)

Possibility of funding:

To be discussed

Possible continuity with PhD:

To be discussed

Comments:

Recent publications: - doi: https://doi.org/10.1101/2024.01.21.575681 - doi: https://doi.org/10.1101/2023.08.07.552350 - Weng C, et al. "The energetic and allosteric landscape for KRAS inhibition" - Nature 2024 Previous master thesis project: - Lindeboom RG, et al. "The rules and impact of nonsense-mediated mRNA decay in human cancers" - Nature Genetics 2016