Project Plan

Master's Project Plan

Uncovering RNA Off-Target Binding Preferences for Improved Antisense Oligonucleotides

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Project Details

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ECTS Points:
The project spans 30 ECTS points

Project Period:
11.05.2022 - 11.10.2022

Abstract

For this project, antisense oligonucleotides (ASOs) targeting RNA will be analysed for their propensity to elicit off-target effects. Upon binding to the target RNA, the ASOs recruit endogenous ribonucleases that cleave the RNA. The ASOs are often designed to bind perfectly to their intended targets, but can also bind imperfectly to off-target RNA. ASOs with too severe off-target effects cannot be developed as therapeutics. In this project, there will thus be a focus on discovery to what extent the ASOs bind off-target RNA, as well as why this happens, and what the characteristics of imperfect binding are. This could allow for designing more selective ASOs for future use.
Relevant data has already been created by Roche and publicly available data will be used to supplement, to allow for a larger scale of analysis. The data consists of RNA-Seq analyses of transcriptomes in cells and tissues treated with ASOs at various concentrations. The

project is thus purely focused on data analysis and exploration, which will be carried out programatically, using R and/or Python. The project is done in collaboration with Roche Innovation Center Copenhagen (RICC)

The expected outputs of the study are: (1) A thesis report. (2) Results from statistical analysis of the data giving insight into what contributes to off-target binding. (3) If time and results permit, a computer model which can predict ASO off-targets.

Approach

The approach to the project has four main focus points. To complete these four steps, programming in either R or Python will be utilised.

1. The first step is to gather data. Whilst Roche has already created a data set, more RNA-Seq data will be found publicly to supplement. This entails screening assorted sources of RNA-Seq data to find relevant and useful data. Primarily, it is important, that the nucleotide sequence and the modifications done to the ASOs is available for each data set. It is also important, that the transcriptome is analysed after addition of only a single ASO, and not a mix of these, as that would complicate the interpretation of the data.

2. The second step is the process the data. RNA-Seq data will often be available as raw sequence reads or raw count data, and this will need to be processed to allow for interpretation of which genes are down-regulated following addition of the ASOs. This step will thus consists of RNA-Seq pipelines, taking the data from raw reads or counts to a set of differentially expressed genes.

3. After the data has been properly formatted and processed, sequence analysis can begin. The ASOs will be mapped to where on their off-target RNA they could possibly bind. As this is not experimentally confirmed, it will be a matter of finding the most probable binding-site on the RNA.

4. Finally, based on the ASOs and their most probable binding sites on the off-target RNA, it will be determined whether or not there are any similarities between the imperfect bindings, and thus whether any rules for imperfect binding can be formulated.

5. If possible, this step will include the potential creation of a model able to predict ASO off-targets based on its sequence.

Timeline

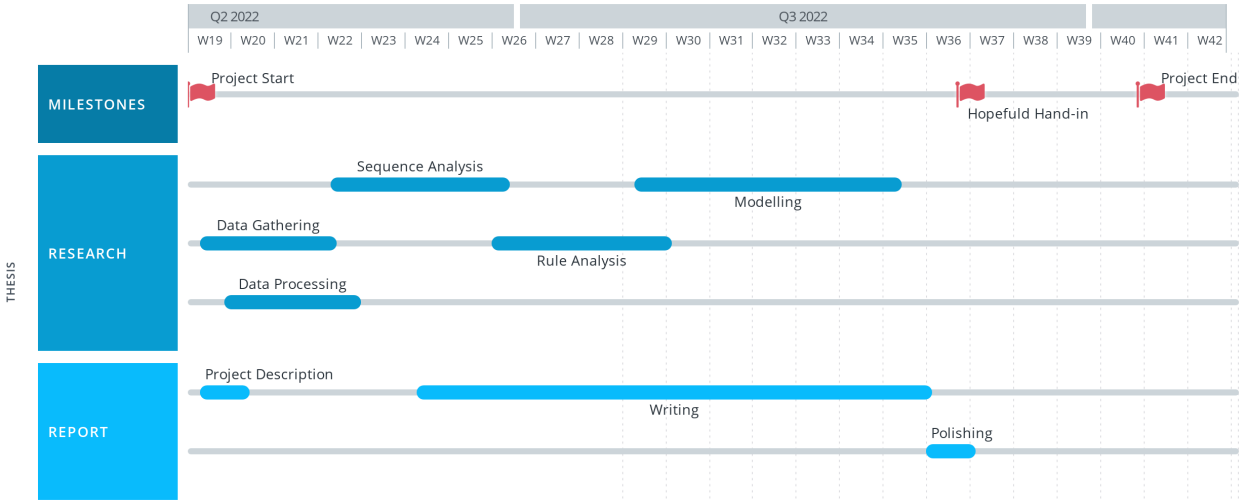

Figure 1: *Gantt Chart for the Project*

If time permits, the steps of 'Data Gathering' and 'Data Processing' may be repeated through the project, if more data is deemed necessary for a more substantial analysis.

Learning Objectives

A student who has met the objectives of the project will be able to:

- Explain how antisense oligonucleotides are created and utilised as therapeutics
- Discuss strengths and weaknesses for treatment purposes
- Explain the physical and chemical processes being binding of antisense oligonucleotides to RNA, and RNase H binding to the ASO-RNA duplex and the subsequent cleavage and degradation of RNA
- Gather and argue for use relevant data from both internal and external sources
- Successfully use RNA-Seq pipelines to go from raw data to properly processed data
- Analyse and attempt to predict off-target effects. Explain and discuss results.
- Discuss and conclude upon the results of the analyses of the off-target effects of oligoucleotides
- Convey technical information, theory and results both written, visually, and orally
- Gather and interpret technical information and master technical problem solving
- Find the most informative way of illuminating the problem by critical information search and critical gathering of new knowledge