iso-CRISPR: Dissecting isoform functionality

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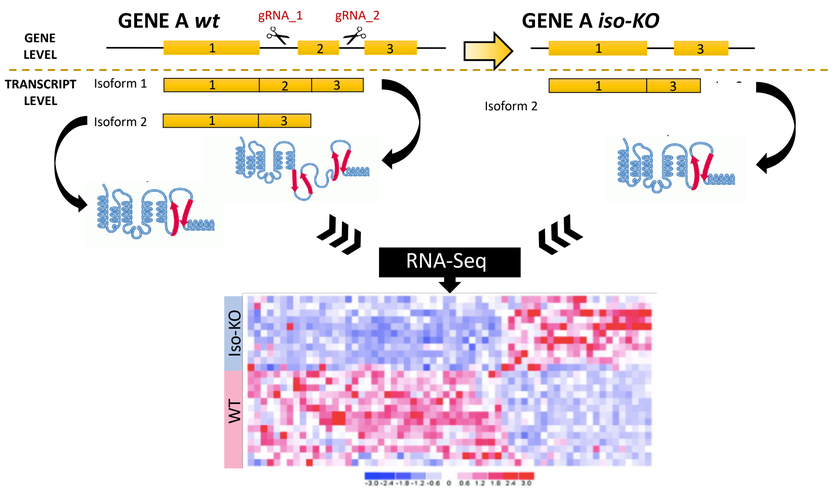
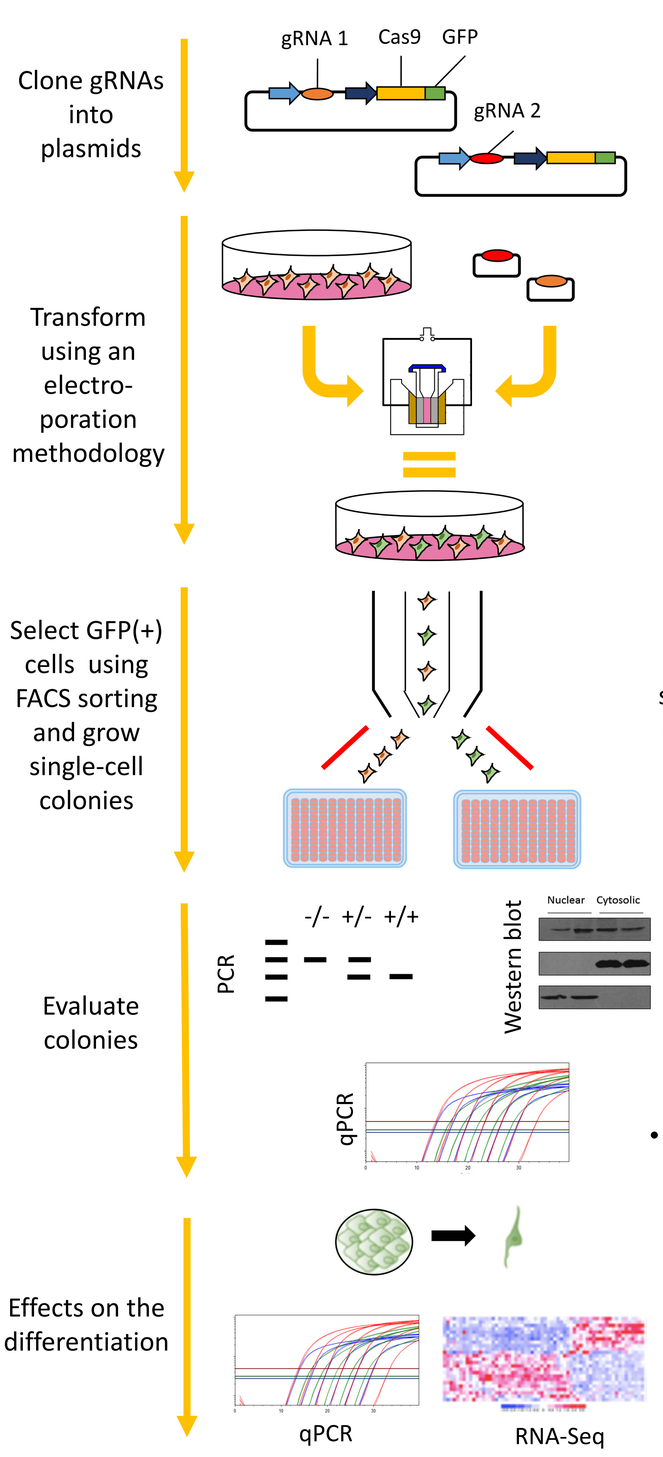
Introduction

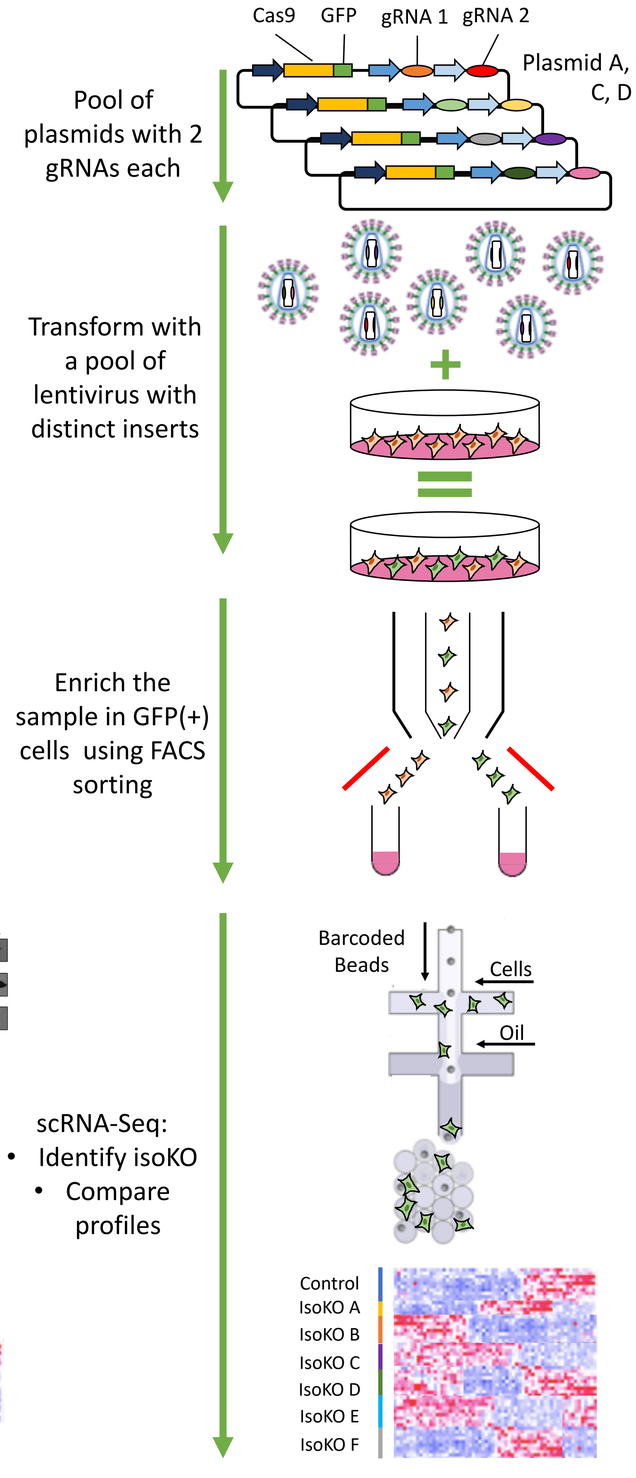
Eukaryotic cells are able to obtain several transcripts from the same gene thanks to the Alternative Splicing (AS) during the mRNA maturation process. Even though this is a well known fact, proteomic approaches failed in finding evidences of AS at the whole-genome level. In consequence, there is a lively debate about the importance of the AS and its contribution to the proteome diversity. Our **hypothesis** is that, if an alternative isoform has a relevant function, its elimination from the system will impact in the transcriptomic profile of the deletion mutant. Using a pair of gRNAs and CRISPR/Cas9 system, we would remove a differentially spliced exon at the genomic level, preventing its expression. During this project we intend to develop an assay that will allow us to **screen** the functionality of a high number of alternative isoforms in a systematic way using **CRISPR/Cas9** and **single-cell RNA-Seq**.



Phase I



Phase II



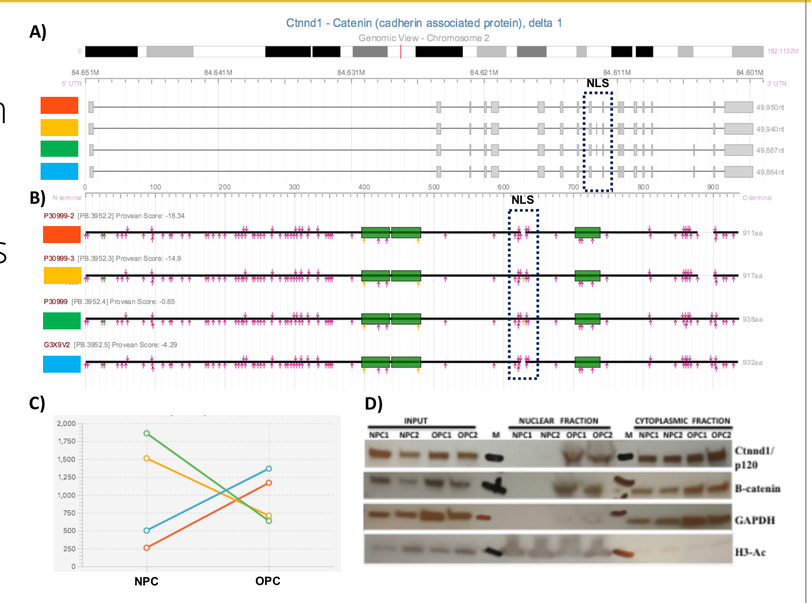
Phase I: Proof of concept

Objectives

- Remove 10th exon from the genome using CRISPR
- Select GFP(+) cells
- Obtain NPCs colonies iso-KO for some isoforms of Ctnnd1

Questions to answer

How important is that Ctnnd1 is present in the nucleus? Does it have an impact over the whole transcriptome? Is it affecting the differentiation into OPC?



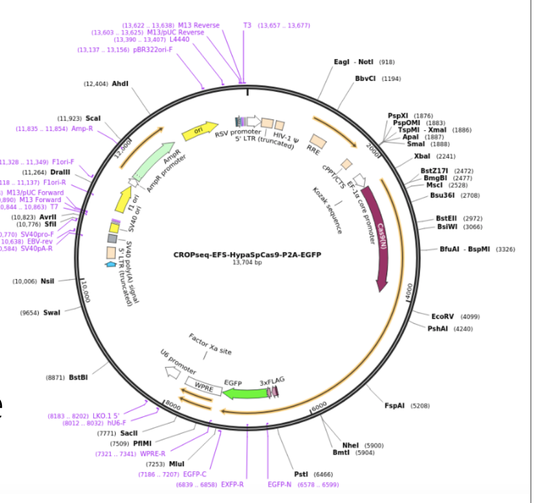
Phase II: High-throughput iso-KO

Objectives

- Paired cloning
- Multiple transformation
- gRNAs identification
- scRNA-Seq profiling

Questions to answer

How many of the isoforms have a significant role for the cell? Can we distinguish mutations over the same gene? Can we ensure that AS is increasing the proteome complexity and has a functional effect?



Innovation

- Sequence the transcriptome of NPCs at the single-cell level.
- Address functional impact of isoforms through CRISPR/Cas9 gene editing.
- Coupling of scRNA-Seq and double-guided CRISPR/Cas9.
- Evaluation of hundreds of iso-KO simultaneously.
- Development of bioinformatic tools to refine the association between the gRNA introduced and the caused mutation.

Expected Results

- A robust procedure to screen the function of several isoforms in an arrayed manner.
- Add significant and new information about the role of Alternative Splicing in cellular biology.
- Validate *in silico* predictions of functional differences between isoforms.
- Identification of relevant isoforms for the differentiation process of neural progenitor cells into oligodendrocytes.