Characterization & Comparison of CRISPR-Derived Editing Methods for Human Pluripotent Stem Cells (hPSCs)

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Research Question

What are the advantages and disadvantages of each CRISPR-derived system, and which systems are optimal for specific engineering applications of hPSCs?

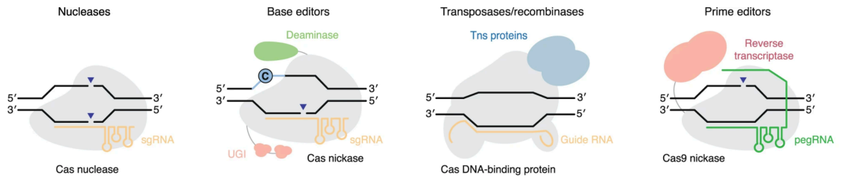

Figure 1. Visual overview of the four classes of CRISPR-derived systems. Source: Anzalone et al. 2013 [3].

Introduction

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system is a vital tool for modifying genomic DNA. When used in conjunction with human pluripotent stem cells (hPSCs), CRISPR enables researchers to perturb gene function, model diseases, and develop therapeutics [2].

Initial CRISPR systems utilized a Cas9 endonuclease and sgRNA to generate double-stranded breaks at a target loci. The breaks are then repaired with either non-homologous end joining (NHEJ) or homology-directed repair (HDR) [2]. Since then, the technology has been modified for specific applications. There are currently four classes of CRISPR-derived editing agents: nucleases, base editors, transposases/ recombinases, and prime editors (Figure 1) [3]. Each class has its strengths and limitations, and many factors (such as the edit type) should be considered when choosing an editing agent [3]. The purpose of this project is to create a guidebook for determining the most optimal editing method for different engineering applications. Furthermore, the feasibility of each class will be evaluated when employed on hPSCs.

Methods

Research data was collected from multiple peer-reviewed literatures that utilized CRISPR technology and hPSCs. Then, the data was organized based on the editing agent class and the intended gene editing application.

To conduct the data analysis, a list of metrics were generated to evaluate the viability of each class. For each editing objective (generating indels, mutations, etc. of varying size), the methods were ranked from most to least optimal (or not applicable) based on their editing efficiency, off-target editing, and other metrics.

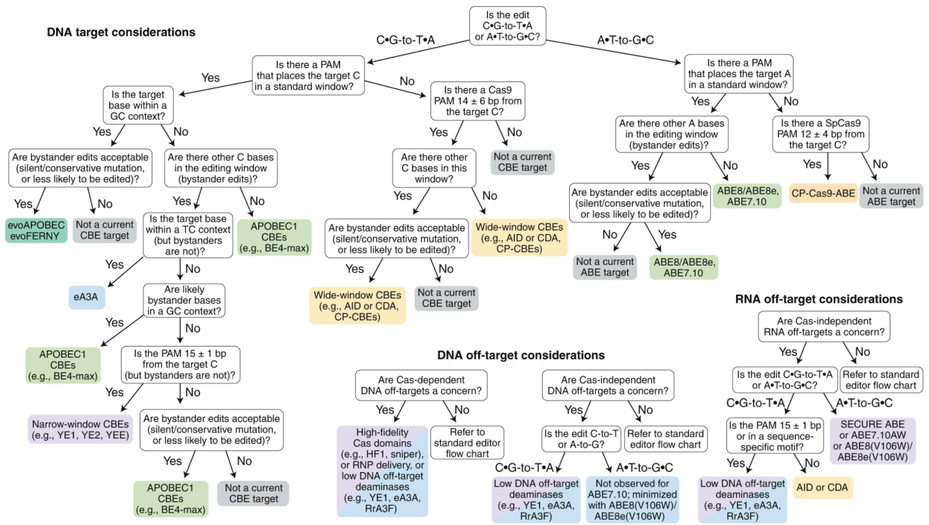

Figure 2. Decision tree for base editors. Source: Anzalone et al. 2013 [3].

Future Work

Each editing agent class has a unique set of limitations that restrict their application. Some metrics, such as the possible edit types and locations, have more impact than others when choosing an editing agent. Therefore, additional research/analysis will be conducted to assign the established metrics different weights based on importance. Then, a decision tree will be designed for each editing objective to help readers select the most optimal process for their experiment. The decisions will be organized so that the initial branches are more crucial to editing success than the later branches.

References

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