

High-Throughput RNA-Targeting Pooled CRISPR Screens and CRISPR-Cas13 Guide Design

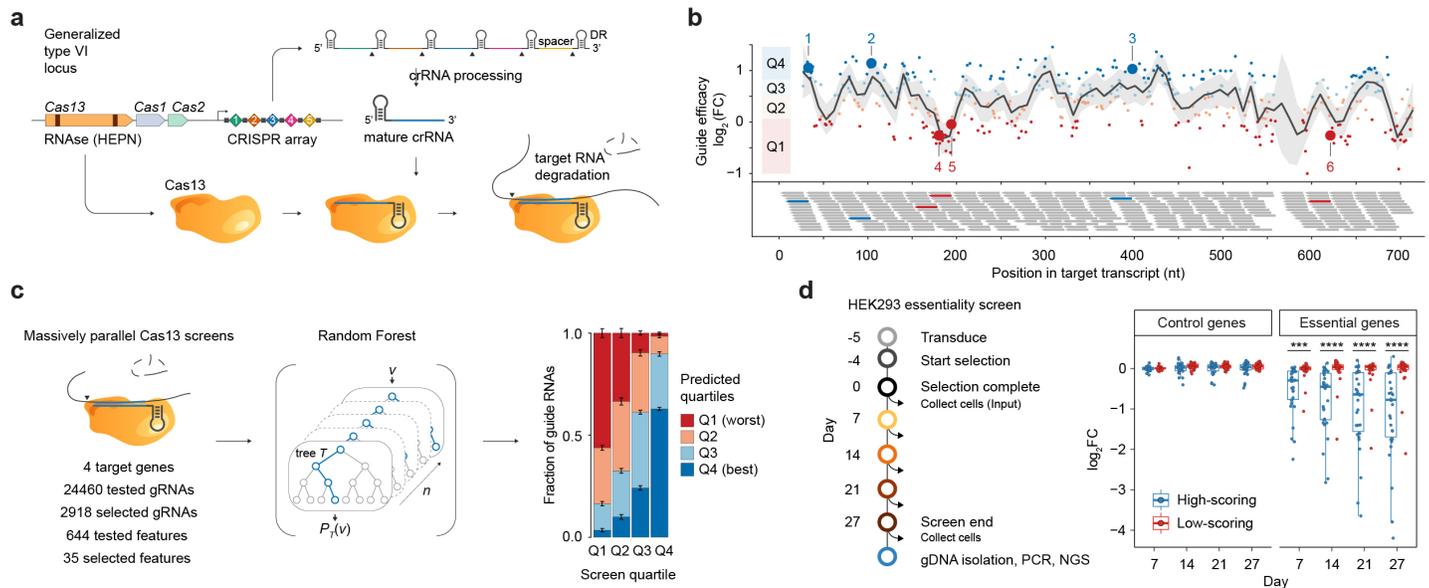


Figure 1. Massively parallel Cas13 screens reveal principles for guide RNA design. (a) Generalized CRISPR type VI loci of RNA-guided RNA-targeting Cas13 proteins. (b) Systematic measuring of guide RNA (gRNA) target knockdown efficacy of thousands of gRNAs tiled along the target RNA. The gRNA efficacy can be grouped into efficacy quartiles (Q1 – Q4), with Q4 gRNAs representing the most effective class. (c) We used machine learning algorithms to learn gRNA feature weights and build a generalizable model to predict gRNA efficacy. (d) Validation of predictive model using forward genetic pooled CRISPR-Cas13 screens. Predicted high-scoring gRNAs targeting essential genes were depleted over time, allowing for the identification of essential genes.

BACKGROUND

The discovery of CRISPR and the development of pooled CRISPR mutagenesis screens has greatly propelled the identification of genes involved in a variety of biological phenotypes. Most CRISPR screens at present are directed at DNA, however many regions of the human genome cannot be edited or targeted by DNA-targeting CRISPR enzymes. Likewise, organisms like RNA viruses (such as coronavirus or influenza) cannot be targeted by typical CRISPR screens. Cas13 is an RNA-targeting enzyme that is guided to its target by CRISPR RNAs and has low off-target effects. Unlike CRISPR-Cas9, little is known about the targeting preferences of CRISPR-Cas13, or the features of optimal Cas13 guide crRNAs. To develop high-throughput RNA-targeting pooled CRISPR screens, there is a pressing need for tools and methods for screening, designing, optimizing, and selecting Cas13 guide RNAs with high specificity and efficiency but low off-target activity.

DESCRIPTION

Scientists in the lab of Dr. Neville Sanjana at the New York Genome Center have developed a new CRISPR screening technology that specifically targets RNA using the RNA-targeting enzyme CRISPR-Cas13. They have engineered an optimized platform for massively-parallel genetic screens at the RNA level in human cells. This screening technology can be used to understand many aspects of RNA regulation and to identify the function of non-coding RNAs. They have also developed a machine learning-based predictive model that allows users to predict the best guide RNAs to target any protein-coding region in the genome, available as an online web tool. Using the model derived from their massively-parallel screens, the researchers have identified optimal guide RNAs that could be used for future detection and therapeutic applications against SARS-CoV-2 (the novel coronavirus that causes COVID-19).

BENEFITS

- High-throughput CRISPR screens with RNA-targeting CRISPR-Cas13
- Robust RNA target knockdown with increased efficacy over existing methods
- Next-generation synthetic Cas13 guide RNA scaffolds for improved knock-down
- Technology that describes features of high-efficacy Cas13 guide RNAs
- Web-based tool for guide RNA efficacy predictions from several common model organisms, including human
- Software to predict optimal RNAs to target any RNA in the human (or other) transcriptome

APPLICATIONS

- Genome-wide CRISPR screens to identify non-coding RNAs of the genome implicated in disease
- Development of therapeutics that target known disease-causing variants without altering a patient's DNA
- Development of Cas13-based therapeutics against RNA viruses such as coronavirus
- Development of next generation biosensors that discriminate between closely related RNA species

PATENT INFORMATION

US Provisional Patent Application #: 62/952,222

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