

NYGC Researchers Develop CRISPR-Based Screen to Target RNA Viruses Like SARS-CoV-2

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NEW YORK – Researchers at the New York Genome Center have developed a CRISPR-based genetic screening platform that uses the Cas13 nuclease to target RNA instead of DNA, providing a new tool for researchers looking to target the genome of the SARS-CoV-2 virus and other RNA viruses.

In a study published on Monday in *Nature Biotechnology*, investigators led by NYGC and New York University researcher Neville Sanjana said they set out to use type VI CRISPR enzymes for gene knockdown screens because of their RNA-targeting activity that enables specific and robust target gene knockdown without altering the genome.

To date, PguCas13b, PspCas13b, and RfxCas13d have been reported as showing high RNA knockdown efficacy with minimal off-target activity, the researchers wrote. They compared the ability of these Cas13 enzymes to knock down green fluorescent protein (GFP) mRNA when directed to either the cytosol or the nucleus, and found that RfxCas13d (CasRx) consistently showed the strongest target knockdown.

To define rules for the design of Cas13d guide RNAs (gRNAs), they conducted massively parallel screens targeting messenger RNAs (mRNAs) of a GFP transgene, and CD46, CD55, and CD71 cell-surface proteins in human cells. In total, the researchers measured the activity of 24,460 gRNAs with and without mismatches relative to the target sequences, and found that knockdown efficacy was driven by gRNA-specific features and target site context.

The investigators then developed a computational model to identify optimal gRNAs and confirm their generalizability, testing 3,979 guides targeting mRNAs of 48 endogenous genes. They showed that Cas13 can be used in forward transcriptomic pooled screens, and that their model could predict optimized Cas13 gRNAs for all protein-coding transcripts in the human genome. They also [developed a web tool](#) for predictive scoring of Cas13 gRNAs.

Importantly, the researchers recently used their gRNA predictive model to identify optimal gRNAs that could be used for future detection and therapeutic applications against the SARS-CoV-2 virus, which causes COVID-19 and has an RNA genome.

In an email to GenomeWeb, Sanjana said he and his colleagues started working on Cas13 targeting of SARS-CoV-2 in late January. Using the publicly available sequence from an early Wuhan patient isolate, they downloaded the entire RNA genome of the virus and designed optimized Cas13 guide RNAs to target

it using the approach described in the Nature Biotechnology study.

"Since coronavirus work cannot be done in our lab due to the pathogenicity of the virus, we also developed a simple reporter system with small pieces of SARS-CoV-2's genome inserted into the fluorescent reporter that we use in the paper," he said.

More recently, he noted, Hans-Hermann Wessels, a postdoc in Sanjana's lab and co-lead author on the study, used the first sequenced coronavirus strain from New York to design guide RNAs customized to a particular strain or isolate. "This is important because the virus does mutate and change over time and so any nucleic acid-based therapy will need to be flexible and re-programmable," Sanjana said.

The team [created a tutorial](#) on how to use the machine learning framework and open-source software to predict Cas13 guides for any target or any virus, including SARS-CoV-2.

The researchers tiled gRNAs across many different transcripts, including several human genes, in order to easily measure transcript knock-down via antibody staining and flow cytometry. They uncovered several biological insights that could expand the application of Cas13 enzymes.

For example, the researchers found that certain regions of the gRNA are more important for recognition of a target RNA. Using thousands of gRNAs with one, two, or three single-letter mismatches to their target RNA, they identified a seed region that is exquisitely sensitive to mismatches between the CRISPR guide and the target. They also found significant differences in protein knockdown when targeting different protein-coding and non-coding elements of mRNAs, and found evidence that Cas13 competes with other RNA-binding proteins involved in transcript processing and splicing.

Since the paper's publication, a couple of public health and drug development groups have contacted Sanjana and his colleagues for possible partnerships to expand on their research, he said, but these efforts are at a very early stage.

"We would welcome partnerships from academia, industry, and funders to help us accelerate our efforts to use Cas13 to combat SARS-CoV-2 and other RNA disease vectors," Sanjana added. "In particular, because SARS-CoV-2 requires extensive biosafety containment, it would be great to work together with labs that already work with the virus and test the efficacy of different CRISPR therapies to block the virus. We also currently do not have funding for any of our Cas13 work and really do need additional support to scale up these efforts."

He did note that for effective Cas13 targeting of coronavirus and other RNA viruses, there are many technical hurdles that need to be addressed, such as picking the right delivery vehicle and finding the optimal format for Cas13. The researchers are planning to use their design pipeline to optimize Cas13 therapies for targeting of several different RNA pathogens, but they've had to transition to virtual lab status due to social distancing.

"We hope to back at the bench as soon as possible to continue this work," Sanjana said.