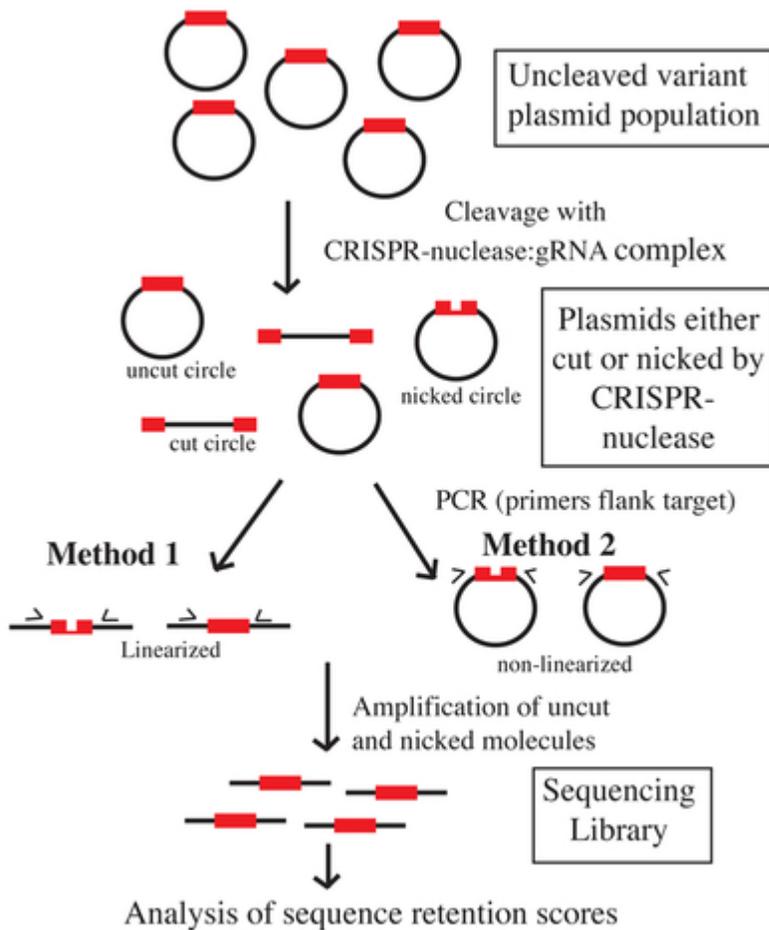


**Docket #:** S17-475

# **Programmed CRISPR-Cas nucleases are potent RNA-guided nickases**

Researchers at Stanford have shown that two CRISPR-Cas nucleases, Cas9 and Cpf1, can be programmed to produce efficient RNA-guided nickases. They were programmed without modification of the nuclease protein using guide RNAs with specific patterns of mismatches to an intended DNA target. While nickases have been used for many biochemical and cell biological tasks, these often involve a modification of the protein enzyme carrying out the recognition and cleavage, which adds to the experimental and operational requirements of using CRISPR systems. An advantage of the present invention is the ability to use a native (non-altered) CRISPR protein and/or to simultaneously carry out nicking and cutting activities using the same enzyme.

**Figure:**



**Figure description:** Schematic of in vitro high throughput plasmid libraries and subsequent steps used to assess representation or each sequence before and after CRISPR-Cas interaction.

### Stage of Development

The researchers have observed efficient nicking activities on specific classes of mismatched DNA targets. To date, the ability of specific guide RNAs to program wild-type (unmodified) CRISPR enzymes to nick at specific sites has been demonstrated both in vitro and in intact yeast cells.

## Applications

- Modifying DNA in vitro (nicking at a specific site) or in vivo (nicking or cutting if two nickases are used)

## Advantages

- Promotes potent nickase activity without mutating Cas9 or Cpf1
- Can simultaneously carry out nicking and cutting activities with the same enzyme
- Method could be adapted to numerous other CRISPR systems that exist in nature.

## Publications

- Becky Xu Hua Fu, Justin Daniel Smith, Ryan T. Fuchs, Megumu Mabuchi, Jennifer Curcuru, Gregory Brett Robb, Andrew Z. Fire, [Target-dependent nickase activities of the CRISPR-Cas nucleases Cpf1 and Cas9](#), Nature Microbiology 2019 May; 4(5):888-897. Published online 2019 Mar 4. doi: 10.1038/s41564-019-0382-0

## Patents

- Published Application: [WO20190213430](#)
- Published Application: [20210163940](#)

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