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RECOMBINANT CRISPR-CAS9 NUCLEASES WITH ALTERED PAM SPECIFICITY

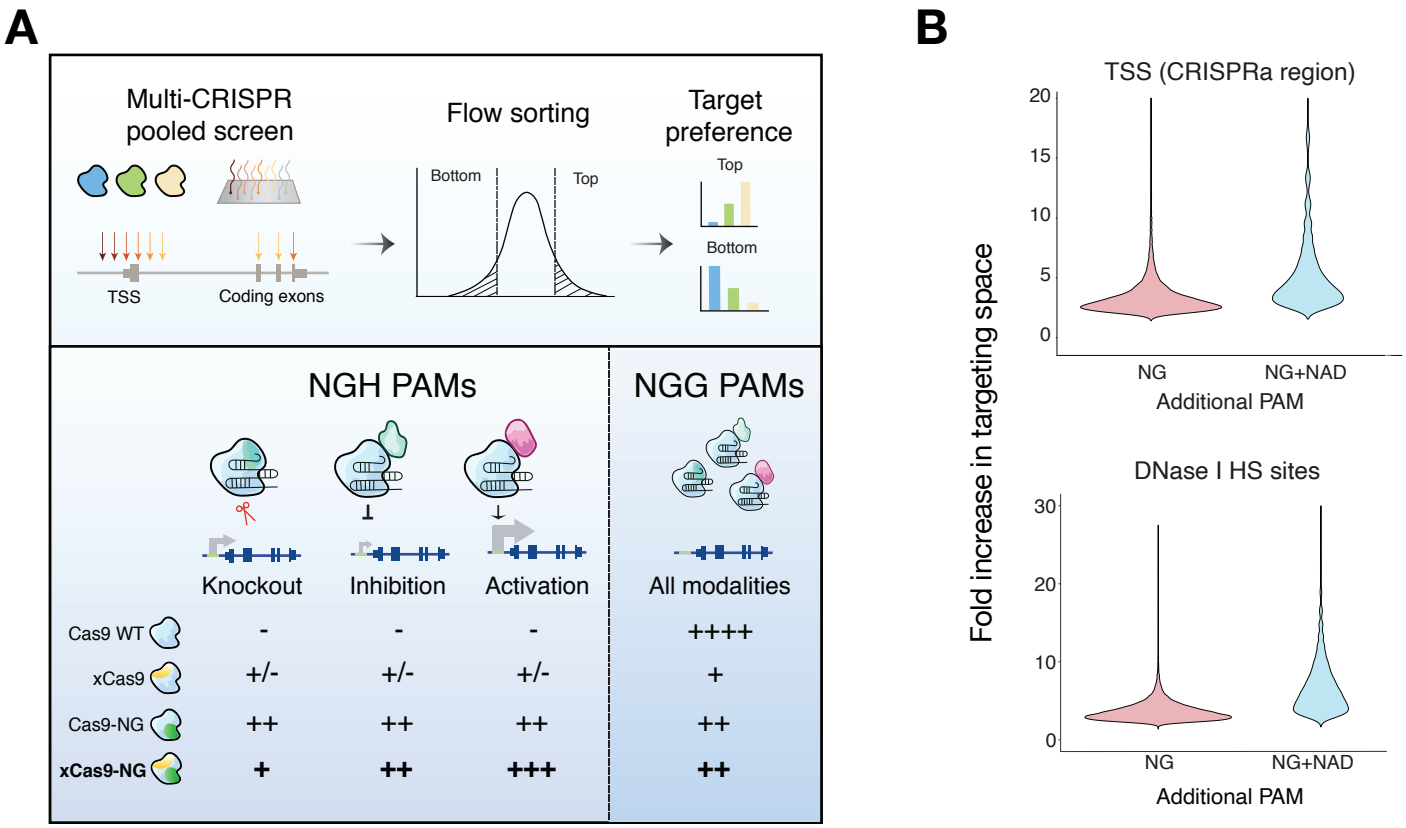

Figure 1. **(A)** Overview of the high-throughput Cas9 comparison screen and a performance summary of tested Cas9 enzymes at different genome editing tasks. xCas9-NG is superior to other PAM-flexible Cas9 enzymes for transcriptional activation (CRISPRa). (**B**) xCas9-NG can access at least 4 times more sequences than wild-type Cas9 at gene transcriptional start sites (TSS) or non-coding functional elements that may be putative enhancers (DNase I hypersensitivity sites) (NAD = N – any nucleotide; A – adenine; D – A, G, or T).

BACKGROUND

Cas9 is a widely-used CRISPR enzyme with various applications in genome editing, transcriptional modulation, epigenome editing, base editing and prime editing. Cas9 however has a strict requirement for a protospacer adjacent motif (PAM) sequence at its target site which limits its utility in certain genome editing applications where precise positioning of Cas9 is essential, such as gene repression/activation or base editing. There is a need for Cas9 enzymes without the strict requirement for a PAM sequence at the target site to enable genome engineering in currently inaccessible regions of the genome.

DESCRIPTION

Scientists in the lab of Dr. Neville Sanjana at the New York Genome Center have developed a novel PAM-flexible Cas9 variant, relaxing the strict requirement for PAM NGG motif at its DNA target. This PAM-flexible Cas9 enzyme (xCas9-NG) has broader utility for genome editing tasks that require precise Cas9 positioning such as transcriptional activation/inhibition, homology directed repair or base editing. xCas9-NG can target the genome not only at sites with a relaxed NG PAM motif but also at sites with another PAM motif (NAD, where D indicates A, G or T). This expanded PAM flexibility translates to an approximately 4-fold greater targeting space in gene promoter/transcriptional start site regions (for CRISPRa/CRISPRi) and in regions harboring noncoding functional elements (for testing potential enhancers). The Sanjana lab has also devised a high-throughput Cas9 pooled competition screen to compare the performance of PAM-flexible Cas9 variants and the wild-type nuclease at thousands of genomic loci and across several modalities.

BENEFITS

- A novel PAM-flexible Cas9 enzyme (xCas9-NG) that utilizes relaxed NG PAMs and novel NAD PAMs for genome targeting
- xCas9-NG can access up to 4 times more sequences in the human genome than wild-type Cas9
- xCas9-NG is superior for transcriptional activation (CRISPRa) than existing PAM-flexible Cas9 enzymes Cas9-NG and xCas9
- A high-throughput method for conducting CRISPR screens with simultaneous readout of the guide RNA and the identity of Cas9 enzyme and effector domain encoded on a specific barcode.

APPLICATIONS

- Genome engineering approaches that rely on precise Cas9 positioning
- The massively-parallel CRISPR competition screen can quantitatively compare different CRISPR enzymes discovered in future
- Targeting transcriptional activators to promoters and other noncoding functional elements that may be inaccessible to wild-type Cas9 for research and therapeutics

PATENT INFORMATION

US Provisional Patent Application #: 62/964,483

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