Design and characterization of compact and precise Cas molecules for treating diseases in patients

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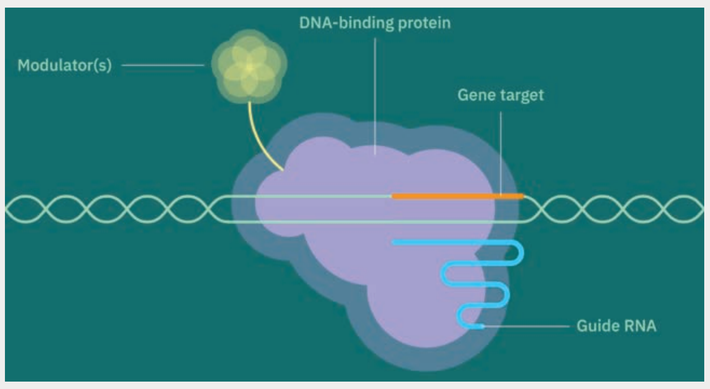
Gene Expression Modulation Systems (GEMs) Platform for epigenetic editing



Epic Bio is at the forefront of epigenetic editing in the development of **highly precise genome engineering molecules for gene therapy that control gene expression** and mitigate and reverse diseases that are beyond the reach of genome editing.

Our engineering efforts have led to the discovery of highly **compact nuclease-deficient dCas enzymes with broad targeting capabilities**, thus enabling efficient delivery by AAV due to their small sizes. Importantly, our novel dCas molecules display flexible PAM requirements and are easily programmed to target therapeutic genes.

Here we present a novel **cellular assay for comprehensive PAM characterization** that faithfully report the PAM requirements of diverse dCas proteins in human cells. These assays enable accurate detection of **greatly expanded PAM profiles for our lead dCas effectors** (dCasONYX, dCasRUBY, dCasTOPAZ), enabling the efficient targeting of disease-causing genes.These assays enable ongoing engineering and characterization of our novel dCas in relevant genomic contexts to facilitate their translation to therapeutics.

Altogether, we present our work to optimize compact and precise Cas molecules at the core of our GEMs epigenetic editing platform and demonstrate their broad utility, representing a major advancement towards treating intractable diseases in patients.

dCas Engineering for Improved GEMs Activity

Combining various semi-rational design approaches including structural comparison, protein stability modeling and sequence alignment from natural diversity, we generated a library of dCas variants to evaluate their performance when incorporated into our GEMs system. A large subset of the library showed improved CRISPRa/i activity and compact size (under 500 aa) when combined with the engineered gRNA scaffold.

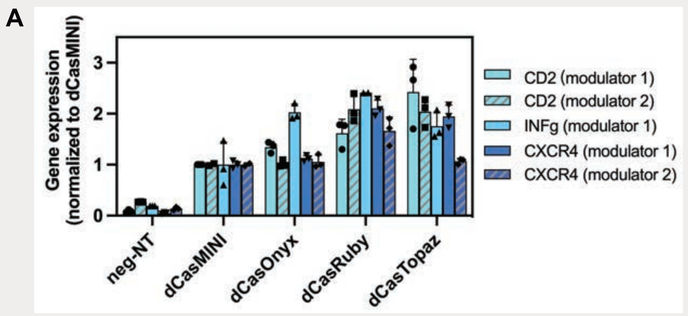


Figure 1 Selected dCas variants showing improved CRISPRa/i activity via transient transfection (above) or stable integration (left).

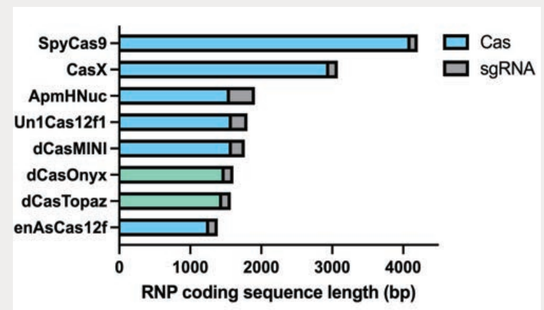


Figure 2 Comparison of coding sequence lengths between various RNPs (Cas effectors and sgRNAs), highlighting compactness optimization in dCasONYX and dCasTOPAZ

Cell-based Assay for PAM Characterization

We developed a cellular assay for quantifying PAM recognition at a single-copy, synthetic locus in the genome. This assay enables direct comparison of PAM recognition between PAM sequences using a reporter of dCas binding. dCas effectors turn on the synthetic reporter (CRISPRa), which can be quantified at the single-cell level.

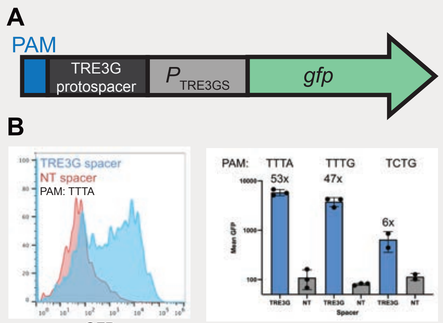
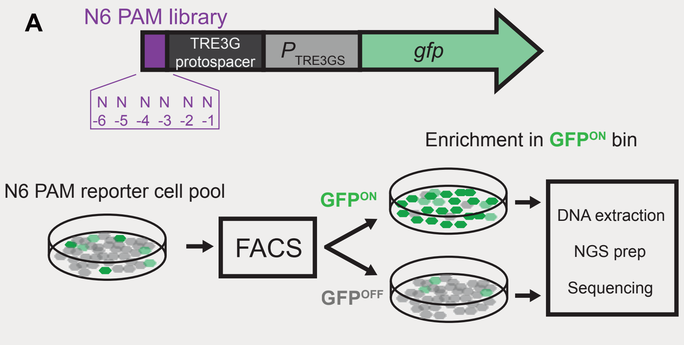
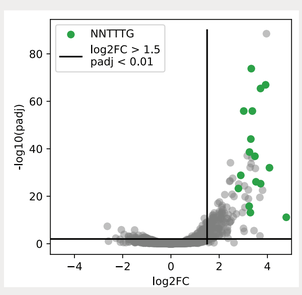


Figure 3 Cell-based CRISPRa assay (arrayed) for quantifying PAM recognition by different dCas variants. **A)** Synthetic reporter for PAM recognition; **B)** Example of selected PAM candidate recognized by dCasONYX

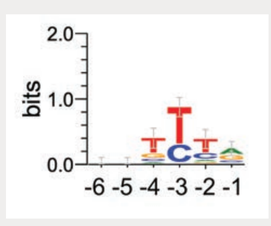
dCasONYX Recognizes a T-rich PAM with Tolerance for C at Position -3

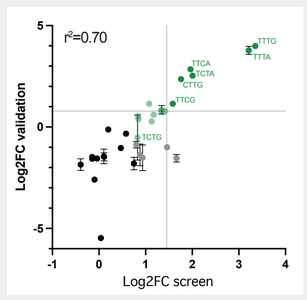
High-throughput pooled CRISPRa screen of dCasONYX against a diverse PAM library (4096 members) provided insight on its unique PAM preference.





4.1% PAM significantly enriched with cutoff set by non-targeting controls





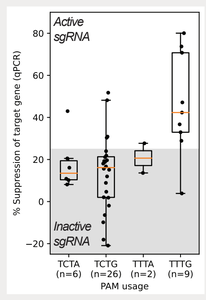


Figure 4 Cell-based PAM characterization screening indicates dCasONYX recognizes a T-rich 4nt PAM sequence with flexibility towards C at position -3. **A)** Schematic illustration of the N6 PAM screening workflow; **B)** Volcano plot showing enrichment (Log2FC) and adjusted p values for each 6nt PAM sequence. Significance thresholds used to define positive hits are shown. Example of all TTTG PAM highlighted in green. Sequence logo of all 6nt PAM hits indicates recognition of a 4nt PAM; **C)** Select PAM were validated in an arrayed screen using the synthetic CRISPRa reporter system; **D)** sgRNA designed to suppress an endogenous gene (CRISPRi) are identified for the indicated PAM, validating their recognition by dCasONYX.

Epic Effectors have Relaxed PAM Recognition, Enabling Broad Targeting Scope

Epic's lead dCas effectors share an expanded PAM profile, enabling high coverage of genomic loci for effective targeting and therapeutic design.

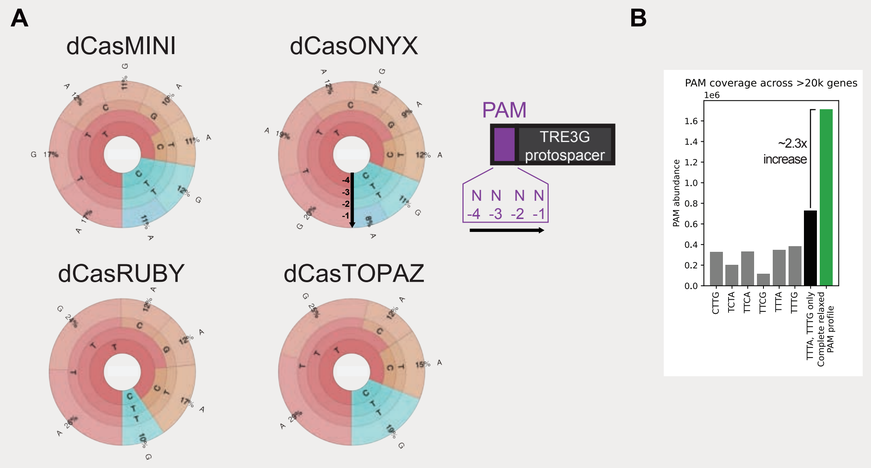


Figure 5 Full PAM characterization of dCasONYX, dCasRUBY, and dCasTOPAZ reveals a shared pattern of relaxed PAM recognition, enabling easy design of therapeutics targeted to many disease causing genes. **A)** Krona plots showing the strengths of PAM recognition for the top 10 PAM identified in each screen, excluding false positives from dCasONYX validation experiments. Sector area reflects the enrichment (Log2FC) from screen. **B)** PAM abundance within optimal targeting window (-1kb to +1kb around TSS) for >20k genes in the human genome.

Summary

Here we present our progress in **rational optimization of nuclease-deficient dCas enzymes for use in gene therapy** by screening for **highly compact and highly active** enzymes.

Further, a novel assay for PAM recognition in human cell lines accurately identifies an **expanded PAM profile for our lead dCas effectors** (dCasONYX, dCasRUBY, dCasTOPAZ). The broad targeting capabilities of these enzymes enables efficient targeting of disease-causing genes in patients.

References and Contact

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2. Takeda, S. N. et al, Mol Cell, 2021, 81, 558.