Engineering of Compact Ribonucleoprotein Complexes for Epigenome Editing

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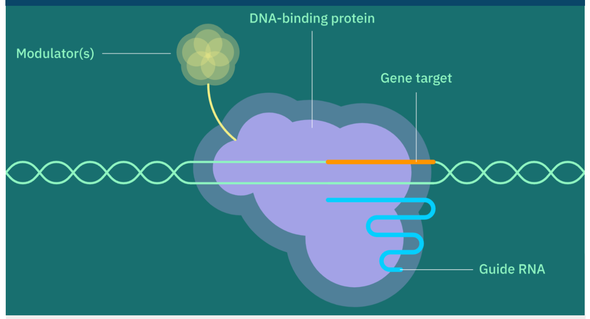
Gene Expression Modulation Systems (GEMs)



Epigenome editing is an emerging new strategy for therapeutic application by activating or silencing target gene expression. At Epic Bio, we have developed a highly optimized CRISPR-based epigenome editing platform termed Gene Expression Modulation systems (GEMs). In this study, we describe the engineered improvement of the gRNA and Cas protein components (Ribonucleoprotein, RNP) of our GEMs platform.

gRNA Scaffold Engineering for Improved GEMs Activity

We have engineered a panel of gRNA scaffolds with reduced size and improved CRISPRa activity.

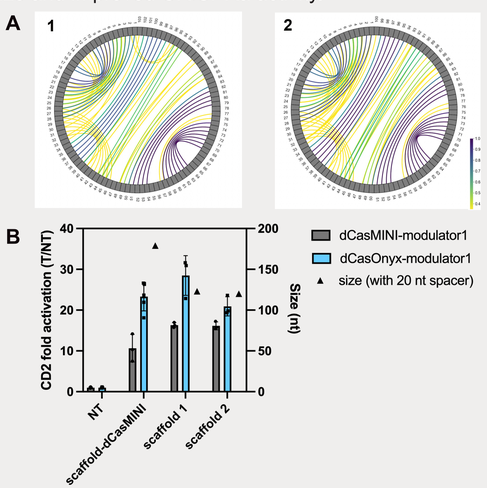


Figure 1 **A)** Selected gRNA scaffold variants (displayed as base pair probability map) and **B)** their CRISPRa activity against endogenous gene.

dCas Engineering for Improved GEMs Activity

Combining various semi-rational design approaches such as 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.

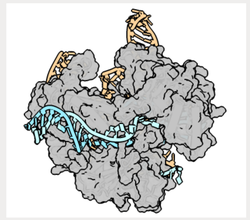


Figure 2 Overview of RNP structure (modeled with an engineered dCas variant and gRNA scaffold)

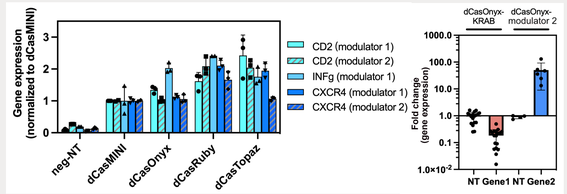


Figure 3 Selected dCas variants showing improved CRISPRa/i activity via transient transfection (left) or stable integration (right)

Spacer Fidelity Characterization Identified Seed Region

Preliminary data indicated a robust seed region in the spacer sequence adjacent to the PAM sequence, with improved fidelity for some of the dCas variants. Larger scale fidelity screen currently in progress.

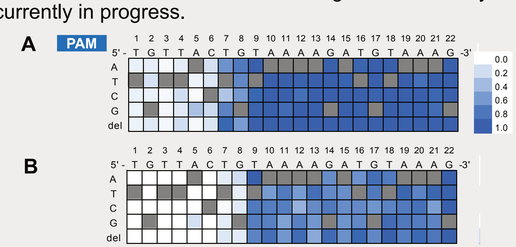


Figure 4 Heat map showing spacer fidelity (CRISPRa activity) of dCas variants fused with modulator2 against a spacer panel containing single mismatch/ deletions, with relative activity normalized to the WT spacer. **A)** dCasOnyx; **B)** dCasTopaz

Cell-based Assay for PAM Characterization

We developed mammalian cell-based assay with synthetic reporter to quantify dCas recognition on individual PAM variants

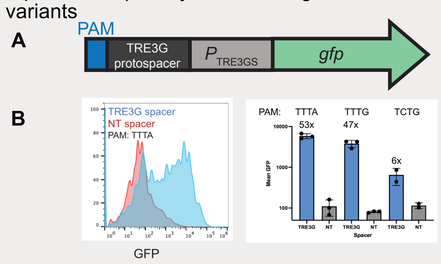


Figure 5 Cell-based CRISPRa assay (arrayed) for characterizing PAM recognition by different dCas variants. **A)** synthetic reporter for specific PAM recognition; **B)** Example of selected PAM candidate recognition by dCasOnyx

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

High-throughput pooled CRISPRa screen of dCasOnyx against N6 PAM library (4096 members) provided insight on its unique PAM preference.

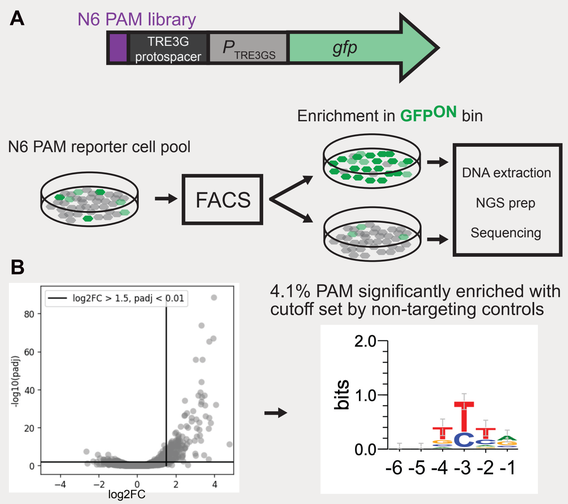


Figure 6 Preliminary cell-based PAM characterization assay indicated dCasOnyx recognizes a T-rich PAM with tolerance for C at position -3. **A)** Shcematic illustration of the N6 PAM screen workflow; **B)** Enriched PAM indicated unique PAM recognition

Summary and Future Direction

In order to improve the epigenome editing efficiency and achieve reduced cargo sizes suitable for delivery via single-AAV, we have engineered a CRISPR-Cas system to reduce its overall size while enhancing the functionality of the ribonucleoprotein (RNP) complex. Through gRNA scaffold and dCas effector modifications, we have achieved a more compact RNP complex that exhibits improved epigenetic editing activity. When fused with the hypercompact transcriptional modulators developed in-house, we were able to demonstrate enhanced precision and efficacy of our epigenome editing system. We also developed a novel pooled cellular assay to sensitively query the PAM profile of these effectors. In summary, we were able to achieve more compact RNP constructs with Cas effectors under 500-amino acid size in complex with gRNA scaffolds around 100-nucleotide long, with relaxed PAM preference as well as enhanced CRISPRa/CRISPRi activity. More in-depth RNP characterization and engineering are in progress.

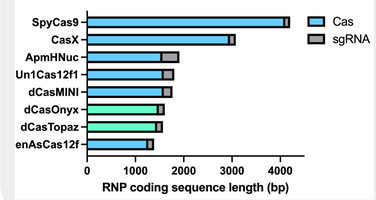


Figure 7 Comparison of coding sequence length between various RNPs

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Poster #80: *EPI-321—A promising CRISPR epigenome engineerig therapy for facioscapulohumeral muscular dystrophy (FSHD)*

Poster #108: *Gene Expression Modulation Systems (GEMS): Optimized CRISPR-Based Epigenome Editing Platform for Versatile Epigenome Modulation*

Poster #100: *Combinatorial screening of transcriptional activation domains for improved activity provides insights into biophysical properties of strong activators*

Poster # 117: Accelerating protein engineering with machine learning—A few-shot transfer learning approach to designing novel gene activators

Poster #71: *Discovery and engineering of hypercompact transcriptional modulators for robust and durable target gene activation*

References and Contact

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Business inquiry: Benson Cheng (benson.cheng@epic-bio.com)

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