TECHNIQUE

CRISPR screens beyond Cas9

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CRISPR-based genetic screens widely used for functional genomics typically involve a Cas9 nuclease and a complex library of guide RNAs (gRNAs) to direct gene-inactivating edits in a high-throughput manner. Two new studies demonstrate the feasibility of functional genomics systems beyond Cas9: a combinatorial DNA editing system involving both Cas9 and Cas12a, and an RNA-targeted system based on Cas13.

In CRISPR screens, defined combinations of target loci can be edited within individual cells by delivering multiple gRNAs expressed from a single viral construct. However, some efficacy limitations have been seen with combinatorial screens using only Cas9 gRNAs. Gonatopoulos-Pournatzis, Aregger, Brown et al. devised Cas Hybrid for Multiplexed Editing and screening Applications (CHyMErA), in which a Cas9 gRNA and a Cas12a gRNA are contained within a single transcript. Cas12a is both a DNA and RNA nuclease; the inclusion of a Cas12a cleavage site within the 'hybrid guide' RNA (hgRNA) allows the Cas12a RNase activity to process the hgRNA into the constituent individual gRNAs.

In mouse and human cell lines stably expressing both Cas9 and Cas12a, the authors showed that combinatorial editing can be achieved in pairs of genes as well as within single genes to generate sizeable gene-fragment deletions. Importantly, the editing efficiency of the Cas9–Cas12a pair was greater than any other tested combination of Cas nucleases.

Although the design rules for efficient Cas9 gRNAs are well established, those for Cas12a are less well known, so the authors generated a library of >40,000 hgRNAs targeting 450 core essential genes in mouse and human cells. Potency was determined based on the degree of depletion of these hgRNA constructs over time in culture (representing loss of cell viability), and a deep learning neural network identified sequence features of the hgRNAs conferring maximal efficacy. The authors have provided a web resource for optimal guide designs (CHyMErA CRISPR tools).

The team then performed combinatorial screens using optimized library designs to investigate distinct biological phenomena. They confirmed known genetic interactions between pairs of genes, such as between *TP53* and its negative regulators *MDM2* and *MDM4*. Additionally, they identified pairs of gene paralogues with functional redundancy (that is, the cellular effect of gene ablation is largely absent until both genes are inactivated). Focusing on genes that modulate the response to the mTOR inhibitor Torin1, they showed that CHyMErA-based double targeting of the coding regions of individual genes is more potent than single targeting. Finally, by targeting pairs of sites within introns, the authors performed a deletion screen for 2,157 exons, identifying alternative exons that affect cell fitness.

In addition to its versatility in functional genomic applications, an advantage of CHyMErA is that using two different Cas nucleases with different sequence requirements affords a much greater range of target sites. However, it remains to be seen whether co-expressing Cas9 and Cas12a causes unwanted perturbations of cell physiology.

In a separate study, Wessels, Méndez-Mancilla et al. investigated gRNA designs for Cas13, an RNA-targeting nuclease with the potential for use in screens based on gene knockdown through transcript cleavage.

Starting with human HEK293 cells expressing nuclear-localized Cas13 and a destabilized *GFP* gene, they screened 7,500 gRNAs tiled across *GFP* and assessed knockdown efficiency by fluorescence-activated cell sorting (FACS). Machine learning approaches identified the sequence features that were indicative of highest gRNA potency.

The team further trained their model for optimized gRNA design by screening additional tiling gRNA libraries targeting the endogenous cell-surface markers CD46, CD55 and CD71 (with knockdown assessed by FACS). As validation, optimized gRNAs largely outperformed non-optimized gRNAs in viability screens using libraries of gRNAs targeting essential genes.

The authors have provided optimized Cas13 gRNA designs for all GENCODE-annotated human protein-coding transcripts in a Web resource (Cas13design), enabling users to adopt these designs in their own screens.

These studies illustrate the expanding options for CRISPR-based screening strategies in diverse biological systems.

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ORIGINAL ARTICLES Gonatopoulos-Pournatzis, T. et al. Genetic interaction mapping and exon-resolution functional genomics with a hybrid Cas9–Cas12a platform. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-020-0437-z (2020) | Wessels, H.-H. et al. Massively parallel Cas13 screens reveal principles for guide RNA design. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-020-0456-9 (2020)

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