GENETIC SCREENS

CRISPR screens come into sight

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A new mammalian genetic screening strategy demonstrates the feasibility of combining CRISPR libraries with in situ sequencing to read out both complex cellular phenotypes and genetic perturbations using microscopy.

CRISPR-based genetic screens have typically been carried out in one of two formats. In arrayed screens, each CRISPR-based perturbation occurs in a separate well of a multi-well plate. Although complex phenotypic profiling is possible by microscopy, the need to process many multi-well plates hinders upscaling to large CRISPR libraries and introduces batch effects. A practically simpler approach is a pooled screen, whereby a mixed CRISPR library is delivered en masse to a population of cells. Although highly scalable to genome-wide throughput, the types of phenotype analysed are fairly simplistic: typically, pooled screens use standard high-throughput sequencing to analyse shifts in the representations of CRISPR perturbation constructs following a simple selection step, such as one based on cell survival or expression levels of a reporter gene.

Feldman, Singh et al. sought to combine pooled screening with

microscopy-based phenotypic profiling. The primary challenge was to identify the genetic perturbation responsible for a cellular phenotype of interest. To achieve this aim, the team devised a two-step microscopy procedure whereby high-content phenotypic characterization is followed by fluorescence-based in situ sequencing to determine the particular CRISPR construct in each cell.

The authors optimized their in situ sequencing approach using different CRISPR library types, showing that CRISPR perturbations could be identified by sequencing the expressed guide RNA (gRNA) itself. However, sensitivity was enhanced when the team designed a CRISPR library in which each gRNA was paired with a 12-nucleotide barcode sequence, and they instead sequenced the expressed barcode. As barcode sequences are fully customizable, the authors designed them in an error-robust way, so that even two sequencing errors would still allow each barcode to be uniquely identified.

In further initial testing, the authors showed that the barcoded screening set-up could identify positive-control CRISPR perturbations that resulted in expression of a tagged H2B reporter protein.

For the remainder of the study, the team applied CRISPR libraries to characterize the genetic determinants of nuclear factor- κ B (NF- κ B) regulation. They used a barcoded CRISPR library of 3,063 gRNAs targeting 963 genes. They visualized >3 million HeLa cells to identify gRNAs that altered the nuclear import of a fluorescent NF- κ B subunit following stimulation with IL-1 β or TNF. Overall the screen

identified known regulators of NF- κ B activation and pinpointed novel NF- κ B regulatory functions for several ubiquitylation-related genes. Repeating the screen across three human cell lines — this time monitoring the localization of endogenous NF- κ B through antibody staining — revealed which genes had more-universal effects on NF- κ B activation versus those that were cell-type dependent.

In follow-up studies, the team further leveraged the strengths of microscopy-based phenotyping. On re-analysis of the initial NF- κ B screen, the hits could be classified according to collateral effects on cellular morphology: for example, disruption of proteasome genes caused a rounded cell morphology with enlarged nuclei. Additionally, the authors performed a live-cell imaging screen, serially visualizing NF- κ B localization, which allowed a more thorough analysis of genes with subtle or time-dependent effects on NF- κ B activation. For example, they identified the MED12 and MED24 subunits of the Mediator transcription complex as negative regulators of NF- κ B activation, and subsequent RNA sequencing (RNA-seq) of MED12- and MED14-deficient cell lines indicated that these Mediator components are involved in restricting the transcriptional response to pro-inflammatory NF- κ B signalling.

This study thus demonstrates a powerful approach to genetic screening of complex phenotypes that is likely to be applicable to diverse screening settings. Future directions include determining whether the system can expand to genome-wide scale, as well as the potential for combining with omics readouts, such as spatially resolved single-cell RNA-seq.

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ORIGINAL ARTICLE Feldman, D. et al. Optical pooled screens in human cells. *Cell* **179**, 787–799 (2019)

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