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TECHNIQUE

A mosaic of enhancer function in single cells

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With more than 2 million predicted enhancers interacting in unique genetic and epigenetic contexts, genome-wide functional assessment of these regulatory elements has lagged behind identification. To overcome this issue, a study in *Molecular Cell* reports the development of Mosaic-seq — short for mosaic single-cell analysis by indexed CRISPR sequencing — which analyses at the single-cell level changes in the transcriptome mediated via targeted enhancer repression.

The strategy uses a catalytically inactive Cas9 (dCas9) fused to KRAB, a strong repressor of enhancer function, and a barcoded single guide RNA (sgRNA) library to generate a mosaic of cells with targeted epigenetic alterations resulting in differing gene expression. Phenotypic characterization was carried out by single-cell RNA sequencing (RNA-seq), and the single-cell transcriptome profiles were correlated with the enhancers targeted by the barcoded sgRNAs.

To validate their approach, the group targeted the β-globin locus in K562 cells. Recruitment of dCas9–KRAB to the enhancer HS2 led to repression of gene expression of its downstream targets *HBG1*, *HBG2* and *HBE1* to a similar degree to that achieved if the promoters of these genes were targeted directly.

To ensure high quality at large scales, the team generated gold-standard data sets using a TNFα stimulation system in K562 cells using both single-cell and bulk-cell RNA-seq approaches. By examining the distribution of gene expression changes, Mosaic-seq could be used for 'virtual fluorescence-activated cell sorting', with the ability to use as few as 300 cells to detect differential expression of low and moderately expressed genes.

After validation, the researchers applied Mosaic-seq to the functional interrogation of 71 constituent enhancers from 15 super-enhancers, asking whether each constituent enhancer contributes equally or whether a subset contributes more to gene regulation. With a focus on DNase I-hypersensitive sites in the β-globin locus control region, the team were able to confirm previous results that the enhancer HS2 is the major contributor to gene expression. Moving to super-enhancers flanking the gene *PIM1*, the team could identify those DNase I-hypersensitive regions that contribute to most gene expression.

Using the Mosaic-seq data set, the researchers were able to investigate general features of enhancers. Looking at epigenetic features of active enhancers in more than 500 chromatin immuno-precipitation followed by sequencing (ChIP–seq) data sets, the team noted that the histone acetyltransferase p300 and RNA polymerase II, along with the sequence-specific transcription factors TAL1 and GATA-2, were enriched at KRAB-responsive enhancers. Moreover, the authors built a statistical model (based on the single-cell penetrance and gene expression contribution of an enhancer) that reflects endogenous enhancer usage.

Finally, Xie *et al.* wondered what role might be played by constituent components of super-enhancers that have little influence on gene expression. To explore this question, combinations of sgRNAs were used to target multiple constituents of super-enhancers simultaneously. The repression of multiple individual components was shown to be significantly greater than that of individual constituents, suggestive of a compensatory relationship between weak enhancers.

Whereas techniques exist that merge single-cell RNA sequencing with genome editing, such as Perturb-seq and CRISP-seq, the analysis of enhancers is hampered by the small number of target genes for each enhancer. Although Xie *et al.* acknowledge the limitations of their system — such as whether KRAB is a universal repressor — Mosaic-seq enables genome-wide functional analysis of enhancers and super-enhancers with an unbiased, scalable and combinatorial single-cell approach.

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ORIGINAL ARTICLE Xie, S. *et al.* Multiplexed engineering and analysis of combinatorial enhancer activity in single cells. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2017.03.007 (2017)
FURTHER READING Burgess, D. J. *et al.* Combining CRISPR perturbations and RNA-seq. *Nat. Rev. Genet.* **18**, 67 (2017)

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