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CRISPRa tiling screens

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Andrea Daniel: This protocol was adapted from the work of Sean McCutcheon and colleagues in the Gersbach lab at Duke University.

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We use this protocol and it's working

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Abstract

This protocols describes methods for characterizing the activity of dSaCas9 as a activator using promoter tiling guide RNA screens in Jurkat cells.

Construction of CRISPRa Jurkat lines

1 Polyclonal dSaCas9^{VP64} and ^{VP64}dSaCas9^{VP64} Jurkat cell lines were generated by transducing Jurkat cells with lentivirus encoding for either dSaCas9^{VP64}–2A–PuroR or ^{VP64}dSaCas9^{VP64}–2A–PuroR.

2 Cells were selected for 5 days using 0.5 µg ml^{-1} of puromycin.

IL2RA CRISPRa tiling screen

3 After selection, 1×10^6 dSaCas9^{VP64} and ^{VP64}dSaCas9^{VP64} Jurkat cells were plated and transduced in triplicate with the *IL2RA* gRNA library lentivirus at a low multiplicity of infection (MOI).

4 Cells were expanded for 10 days, selected for Thy1.1 using a CD90.1 Positive Selection Kit (StemCell Technologies), and stained for Thy1.1 and IL2RA.

5 Transduced cells in the lower and upper 10% tails of IL2RA expression were sorted for subsequent gRNA library construction and sequencing.

6 All replicates were maintained and sorted at a minimum of 500× coverage.

gRNA sequencing

7 Genomic DNA was isolated using Qiagen's DNeasy Blood and Tissue Kit. Genomic DNA was split across 100 µl PCR reactions (25 cycles at 98 °C for 10 s, 60 °C for 30 s, and 72 °C for 20 s) with Q5 2× Master Mix and up to 1 µg of genomic DNA per reaction.

8 PCRs were pooled together for each sample and purified using double-sided (SPRI)bead selection at 0.6× and 1.8×.

9 Libraries were run on a High Sensitivity D1000 tape (Agilent) to confirm amplicon size and quantified using Qubit's dsDNA High Sensitivity assay.

10 Libraries were diluted to 2 nM, pooled together at equal volumes, and sequenced using Illumina's MiSeq Reagent Kit v2 (50 cycles).

11 Primers are available in Supplementary Table 5 of McCutcheon et al. Nature Genetics, 2023. https://doi.org/10.1038/s41588-023-01554-0

Processing gRNA sequencing and gRNA analysis

12 FASTQ files were aligned to custom indexes for each gRNA library (generated from the bowtie2-build function) using Bowtie 2 (ref. **67**).

13 Counts for each gRNA were extracted and used for further analysis in R.

14 Individual gRNA enrichment was determined using the DESeq2 (ref. **68**) package to compare gRNA abundance between groups for each screen.

Protocol references

67. Lagmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012).

68. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).