NOV 30, 2023

Protocol Citation: Andrea R Daniel 2023. CRISPRa tiling screens. **protocols.io** https://protocols.io/view/crispra-tiling-screens-c5rfy53n

MANUSCRIPT CITATION: McCutcheon, S.R., Swartz, A.M., Brown, M.C. *et al.* Transcriptional and epigenetic regulators of human CD8$^+$ T cell function identified through orthogonal CRISPR screens. *Nat Genet* (2023). https://doi.org/10.1038/s41588-023-01554-0

Protocol status: Working
We use this protocol and it's working

CRISPRa tiling screens

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

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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.

Created: Nov 30, 2023

Last Modified: Nov 30, 2023

PROTOCOL integer ID: 91655

Keywords: CRISPRa, dSaCas9, Jurkat cells, IL2RA, tiling screen

Funders Acknowledgement:

NIH

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.