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

TFome CRISPRko screens

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

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ABSTRACT

This protocols describes methods for transcription factor CRISPR knockout screens to reveal cofactors of BATF3 in T cells.

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gRNA library construction

1 The Brunello genome-wide KO[83] library (four gRNAs per gene) was subset for 1,612 TFs[45] and *IL7R*.

2 A total of 550 NT gRNAs were included in the library for a total of 7,000 gRNAs (available in Supplementary Table 6 of McCutcheon et al. Nature Genetics, 2023. https://doi.org/10.1038/s41588-023-01554-0).

3 This gRNA library was cloned into SpCas9 gRNA lentiviral plasmids with either mCherry or BATF3.

gRNA library cloning

4 Oligonucleotide pools containing variable gRNA sequences and constant regions for polymerase chain reaction (PCR) amplification were synthesized by Twist Bioscience.

5 gRNA amplicons were gel extracted, PCR purified and input into 20 µl Gibson reactions (5:1 molar ratio of insert to backbone) with 200 ng of Esp3I digested and 1 × solid-phase reversible immobilization (SPRI)-selected (Beckman Coulter) plasmid backbone.

6 Gibson reactions were purified using ethanol precipitation and transformed into Lucigen's Endura ElectroCompetent Cells.

7 Transformed cells were cultured overnight and plasmids were isolated using Qiagen Midi Kits.

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8 A total of 20×10^6 $CD8^+$ T cells from two donors were activated with CD3/CD28 dynabeads at a 1:1 ratio.

9 At 24 h post-activation, $CD8^+$ T cells were split evenly and transduced in parallel with TFome CRISPRko gRNA libraries with mCherry or BATF3.

10 At 48 h post-activation, cells were electroporated with Cas9 protein. Briefly, the cells were collected, spun down at 90*g* for 10 min, resuspended in 100 µl of Lonza P3 Primary Cell buffer with 3.2 µg Cas9 (Thermo) per 10^6 cells, and electroporated with the pulse code EH115.

11 After electroporation, warm medium was immediately added to each cuvette and cells were recovered at 37 °C for 20 min before being transferred into a six-well plate.

12 On day 3 post transduction, cells were selected with 2 µg ml^{-1} of puromycin for 3 days. On day 9 post transduction, cells were stained for CD8, IL7R and a viability dye.

12.1

Antibody Target	Fluorophore/Sequence	Clone	Isotype	Dilution	Application	Manufacturer	Catalog #
IL7RA	PE-Cy5	eBioRDR5	Mouse / IgG1, kappa	1:100	Flow cytometry	Thermo	15-1278-42
CD8	bv-421	HIT8a	Mouse IgG1, κ	1:50	Flow cytometry	BD Biosciences	740078

13 An SH800 FACS Cell Sorter (Sony Biotechnology) was used for cell sorting and analysis.

14 Cells were then washed with flow buffer, spun down at 300g for 5 min and resuspended in flow buffer for cell sorting.

15 Viable $CD8^+$ T cells in the lower and upper 10% tails of IL7R expression were sorted for subsequent gRNA library construction and sequencing.

16 All replicates were maintained and sorted at a minimum of 75× coverage.