NOV 29, 2023

Protocol Citation: Andrea R Daniel 2023. RNAseq of primary human T cells overexpressing BATF3. **protocols.io** https://protocols.io/view/rnaseq-of-primary-human-t-cells-overexpressing-bat-c5pdy5i6

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

RNAseq of primary human T cells overexpressing BATF3

Andrea R Daniel[1]

[1]Duke University

Andrea R Daniel: This protocol was adapted from Sean McCucheon's work in the Gersbach lab at Duke University.

Andrea R Daniel
Duke University

ABSTRACT

This is a protocol describes methods for performing RNAseq using human CD8+ T cells overexpressing BATF3 or a GFP control.

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

Created: Nov 29, 2023

Last Modified: Nov 29, 2023

PROTOCOL integer ID: 91589

Keywords: RNAseq, CD8+ T cells, BATF3

Funders Acknowledgement:

NIH

Transfections for high-titer lentiviral production

1 Plate 1.2 x 106 or 7 x 106 HEK293T cells in a 6 well plate or 10 cm dish in the afternoon with 2 mL or 12 mL of complete opti-MEM (Opti-MEM™ I Reduced Serum Medium supplemented with 1x Glutamax, 5% FBS, 1 mM Sodium Pyruvate, and 1x MEM Non-Essential Amino Acids).

2 The next morning, transfect HEK293T cells with 0.5 µg pMD2.G, 1.5 µg psPAX2, and 0.5 µg transgene for 6 well plates or 3.25 µg pMD2.G, 9.75 µg psPAX2, and 4.3 µg transgene for 10 cm dishes using Lipofectamine 3000.

3 Exchanged media 6 hours after transfection and collect and pool lentiviral supernatant at 24 hours and 48 hours after transfection.

Primary human CD8+ T cell cultures

4 Isolated $CD8^+$ T cells from individual donors were obtianed directly from vials purchased from StemCell Technologies.

5 Culture T cells were in PRIME-XV T cell Expansion XSFM (FujiFilm) supplemented with 5% human platelet lysate (Compass Biomed), 100 U ml^{-1} penicillin and 100 µg ml^{-1} streptomycin. All media were supplemented with 100 U ml^{-1} human IL-2 (Peprotech).

Transduction of primary human CD8+ T cells

6 Centrifuged lentiviral supernatant at 600*g* for 10 min to remove cellular debris.

7 Concentrate lentivirus to 50–100× the initial concentration using Lenti-X Concentrator (Takara Bio).

8 Transduce T cells at 5–10% v/v of concentrated lentivirus at 24 h post-activation. For dual transduction experiments, T cells were serially transduced at 24 h and 48 h.

RNAseq

9 T cells overexpressing either BATF3 or GFP were cultured for 10 days post transduction.

10 RNA was isolated using Norgen's Total RNA Purification Plus Kit and submitted to Azenta (formerly Genewiz) for standard RNA-seq with polyA selection.

11 Reads were first trimmed using Trimmomatic[72] v0.32 to remove adapters and then aligned to GRCh38 using STAR v2.4.1a aligner.

12 Gene counts were obtained with featureCounts[73] from the subread package (version 1.4.6-p4) using the comprehensive gene annotation in Gencode v22.

13 Differential expression analysis was determined with DESeq2 (ref. [68]) where gene counts are fitted into a negative binomial generalized linear model and a Wald test determines significant DEGs.

DESeq2 results of RNA-seq analyses with BATF3 OE and ZNF217 or GATA3 KO are presented in Supplementary Tables 4 and 7, respectively.

Upregulated and downregulated DEGs were input into EnrichR's GO Biological Processes 2021 database[71] for functional annotation.