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ATAC-seq, primary human T cells overexpressing BATF3

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Andrea Daniel: This protocol was adapted from Sean McCutcheon's work 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 protocol describes methods for performing ATACseq on human HER2 targeted CAR T cells overexpressing BATF3 or GFP. Chromatin remodeling was assessed in acutely and chronically stimulated cells.

Transfections for high-titer lentiviral production

- 1 Plate 1.2×10^6 or 7×10^6 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_{reduced} 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 obtained directly from vials purchased from StemCell Technologies.
- 5 Culture T cells in PRIME-XV T cell Expansion XSFM (FujiFilm) supplemented with 5% human platelet lysate (Compass Biomed), 100 U/ml penicillin and 100 µg/ml streptomycin. All media were supplemented with 100 U/ml human IL-2 (Peprotech).

Transduction of primary human CD8+ T cells

- 6 Centrifuged lentiviral supernatant at 600g 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.

T cell stimulation with HER2+ tumor cells

- 9 HER2+ SKBR3 breast cancer cells were maintained in Dulbecco's modified Eagle medium (DMEM) GlutaMAX supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 1x MEM nonessential amino acids, 10mM HEPES, 100U/ml penicillin and 100ug/ml streptomycin.

- 10 Transfer 1×10^5 HER2 CAR T cells (with or without BATF3 overexpression) to a new 24-well plate with 2×10^5 SKBR3 cells (1:2 E:T ratio) every 3 days (T cells are stimulated on days 3, 6, and 9).
- 11 T cells are removed from antigen stimulation for 2 days to recover after the final round of tumor cell stimulation before ATAC-seq on day 14 after transduction.

ATAC-seq

- 12 Sort a total of 5×10^4 transduced CD8⁺ T cells for Omni ATAC-seq as previously described⁷⁵. See the published Omni ATAC-seq reference protocol: <https://doi.org/10.1038/protex.2017.096>
- 13 Libraries were sequenced on an Illumina NextSeq 2000 with paired-end 50-bp reads. Read quality was assessed with FastQC and adapters were trimmed with Trimmomatic⁷².
- 14 Trimmed reads were aligned to the Hg38 reference genome using Bowtie⁷⁶(v1.0.0) using parameters -v 2-best-strata -m 1.
- 15 Reads mapping to the ENCODE hg38 blacklisted regions were removed using bedtools2 (ref. ⁷⁷) intersect (v2.25.0). Duplicate reads were excluded using Picard MarkDuplicates (v1.130 (ref. ⁷⁸)).
- 16 Count-per-million-normalized bigWig files were generated for visualization using deeptools bamCoverage⁷⁹ (v3.0.1).
- 17 Peak calling was performed using MACS2 narrowPeak⁸⁰ and filtered for $P_{\text{adj}} \leq 0.001$. Peak calls were merged across samples to make a union-peak set.
- 18 A count matrix containing the number of reads in peaks for each sample was generated using featureCounts⁷³ (subread v1.4.6) and used for differential analysis in DESeq2 (ref. ⁶⁸) (v1.136).
- 19 ChIPSeeker⁸¹ was used to annotate the genomic regions and retrieve the nearest gene around each peak.
- 20 HOMER (v4.11) package⁸² was used to find transcription factor binding motifs that contributed to changes in chromatin accessibility with BATF3 OE compared to control cells.



- 20.1 We defined the set of target differentially accessible peaks using DESeq2 ($P_{adj} < 0.05$) and a background set of nondynamic regions ($p \text{ value} > 0.2$ and $|\log_2(\text{fold change})| < 0.2$) with all sets having a sufficiently large number of sequences.
- 20.2 Next, for each set we extracted FASTA sequences from the human reference genome (GRCh38) and ran findMotif.pl to discover motifs and compute the enrichment over background. By default, this function uses a hypergeometric distribution to score motifs to calculate enrichment p-values, controlling for differences in GC-content across target and background sets.

Protocol references

Omni-ATAC-seq: Improved ATAC-seq protocol

<https://doi.org/10.1038/protex.2017.096>

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