Perturb-seq analysis reveals key mediators of TNFα-induced transcriptional response

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Abstract

- To identify genes involved in TNFα induced transcriptional response, a 10X single-cell Perturb-Seq screen was run on HEK293-Cas9 cells with an 88-sgRNA pooled library. As a benchmark, a parallel screen was run with cells transduced in arrayed format with a subset of the sgRNAs of the pooled library.
- To 10X Peturb-Seq screen was carried out in triplicate with increasing amounts of cells/10X reaction, in order to determine:
  a, the minimum number of cells/sgRNA required to cover the full complexity of the pooled library
  b. the maximum number of cells that can be loaded in the 10X reaction without losing single-cell resolution due to encapsulation of cell doublets
- Transcriptional profiling identified TNFRSF1A, IKBKG, RELA, and CHUK as key mediators of TNFα transcriptional response in both the arrayed and pooled library screens, increasing the number of cells per 10X reaction had a positive effect on the sgRNA library coverage, without adversely affecting the magnitude of the observed effect of TNFRSF1A, IKBKG, RELA, and CHUK knockout (KO) on TNFα-mediated transcriptional response.
- Perturb-seq is a promising technology for the efficient and scalable interrogation of many individual gene in one single experiment, enabling the identification of transcriptional profiles linked to specific gene-KOs.

Introduction

Perturb-seq

- Perturb-Seq is a single-cell functional genomics platform that combines pooled CRISPR (sgRNA) genetic screening with single-cell expression analysis.
- Cells transduced with pooled sgRNA libraries are loaded in the 10X reaction which co-encapsulates barcoded beads (each bead a unique barcode) and cells in discrete droplets, most droplets containing one cell and one bead. The sgRNA and mRNAs from each single cell are then labelled with bead barcodes, and used for library construction and the generation of single-cell transcriptional profiles.
- Changes in gene expression associated with specific sgRNAs can be directly linked to specific gene knockouts.

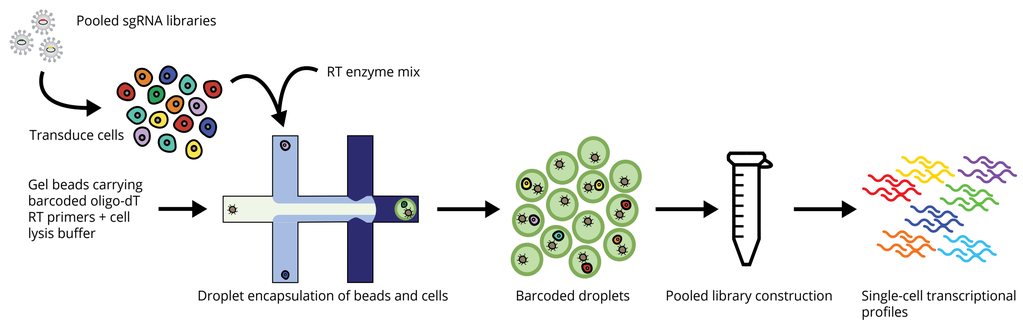

Fig. 1 Perturb-seq workflow

Method

- Tumor necrosis factor-alpha (TNFα) is a proinflammatory cytokine involved in various biological processes including the regulation of cell proliferation, differentiation, apoptosis, and immune response.
- For the Perturb-Seq screen, cells were transduced with the pooled sgRNA library, puromycin selected and expanded for 10 days, then split into 2 samples for 72h TNFα treatment/mock treatment.
- After treatment, cell samples were loaded on the 10x Genomics Chromium Controller according to the manufacturer's specifications at 2,000, 7,500, and 15,000 cells/reaction, and 10X Cell Ranger software was used for data analysis
- For the arrayed screen, cells were transduced with individual sgRNAs, puromycin selected and expanded for 10 days, then split into 2 samples for 72h TNFα treatment/mock treatment
- Genome-wide transcriptome analysis was performed using the DriverMap™ Human Genome-Wide Gene Expression Profiling Assay for the arrayed screen.

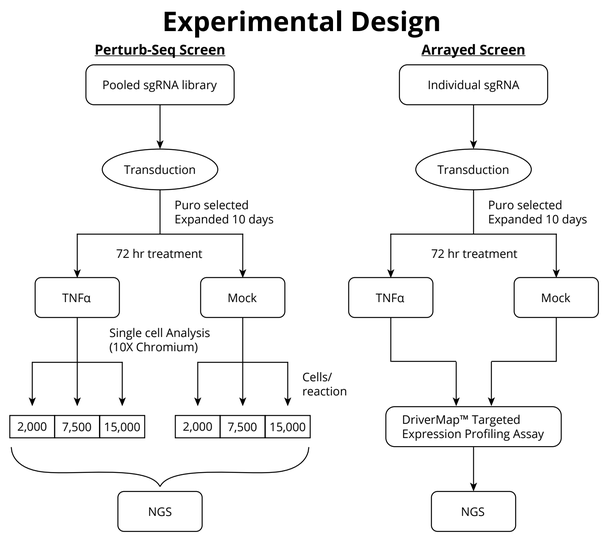

Fig.2 Schematic diagram of experimental design for 10X Perturb-Seq Screen and Arrayed Screen

Results

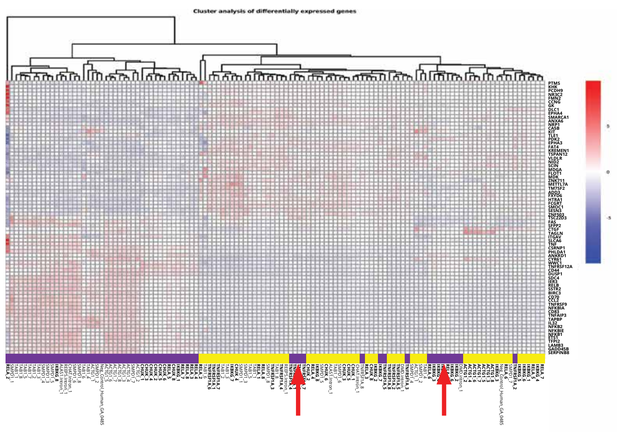

Fig. 3 Arrayed, individual CRISPR-sgRNA screen HEK293-Cas9 cells transduced with individual sgRNA constructs (60 sgRNAs) followed by TNFα treatment (and mock). DriverMap™ Targeted Expression Profiling identified TNFRSF1A, IKBKG, RELA, CHUK as key mediators of TNFα transcriptional response

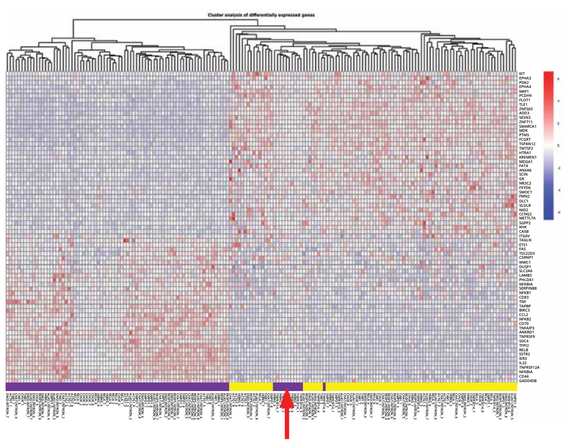

Fig. 4 Perturb-Seq, pooled CRISPR-sgRNA library screen 15,000 cells/10X reaction (1 reaction). 15,000 cells total in one NS500 flow cell

Purple: TNFα treated

Yellow: TNFα untreated

TNFRSF1A, IKBKG, RELA, CHUK

Results (cont.)

Table 1: Perturb-Seq, pooled CRISPR-sgRNA library screen

Cell Number	Cells/sgRNA	Treatment	sgRNA detected
4,000	47	TNFα	57
		Mock	38
7,500	90	TNFα	87
		Mock	87
15,000	180	TNFα	87
		Mock	88

HEK293-Cas9 cells transduced with pooled sgRNA library (88 sgRNAs), followed by TNFα treatment/mock-treatment. Single-cell partitioning was performed on
(a) 2000 cells/10X reaction (2 reactions: 4,000 cells total),
(b) 7,500 cells/10X reaction,
(c) 15,000 cells/10X reaction.

Similar to the arrayed screen, the Perturb-Seq screen identified TNFRSF1A, IKBKG, RELA, CHUK as key mediators of TNFα transcriptional response. **Higher numbers of loaded cells/10X reaction increased the number of detected sgRNAs.**

Fig 5: Effects of gene knockout on TNFα-induced transcriptional activation of IL32 and RELB genes.
IL32 had been previously identified as top transcriptionally activated genes upon TNFα treatment in parental HEK293 cells (data not shown). IL32 and RELB showed again high TNFα-induced transcriptional activation in control-sgRNA HEK293-Cas9 cells in both the arrayed and the pooled library perturbseq screens.
We, therefore, used the activation-fold of these 2 genes to measure gene-KO perturbation of TNFα transcriptional response.

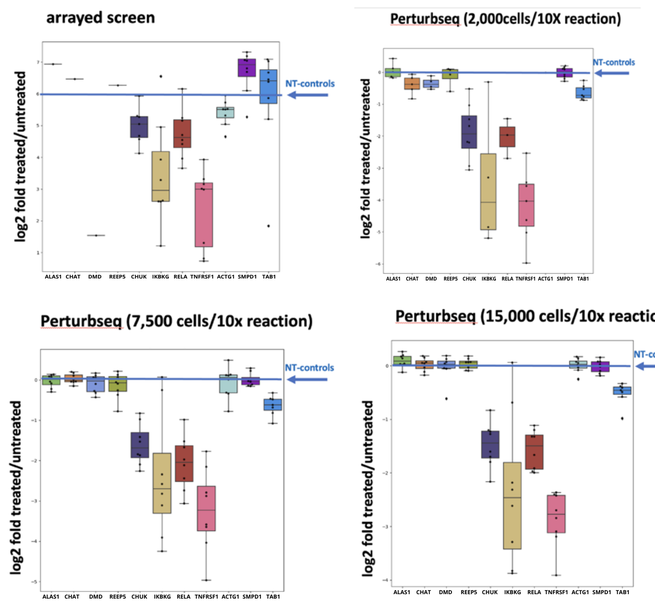

Fig. 5 (a) TNFα-induced Transcriptional activation of IL32

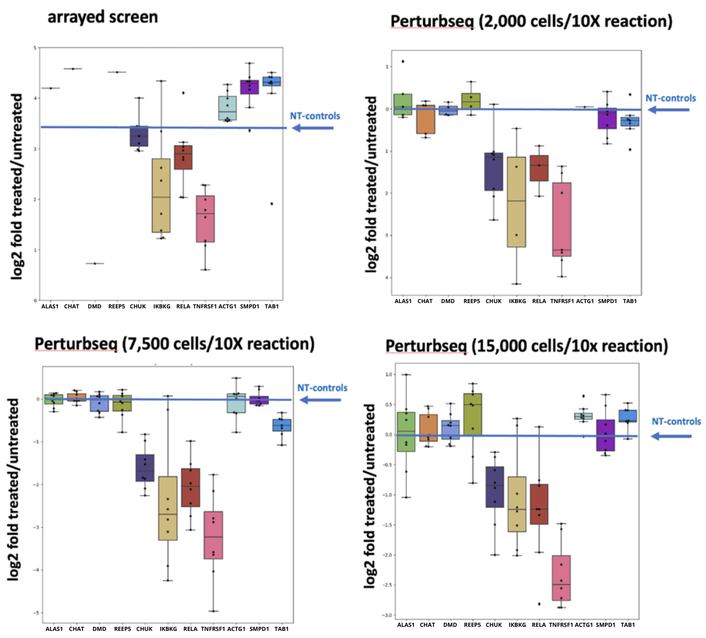

Fig. 5 (b) TNFα-induced Transcriptional activation of RELB

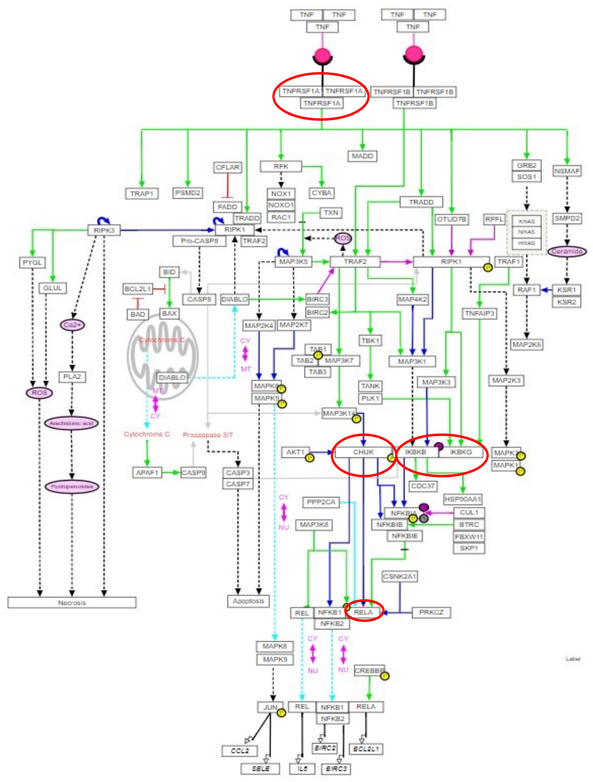

Fig. 6 TNFα mediated signaling pathway

Discussion

- TNFRSF1A, IKBKG, CHUK, and RELA were identified as key mediators of TNFα transcriptional response in both the arrayed and pooled sgRNA library.
- For Perturb screens, increasing the number of cells per 10X reaction from 2,000 (2 reactions: 4,000 cells total) to 7,500 and 15,000 (corresponding to 47 cells/sgRNA, 90 cells/sgRNA and 180 cells/sgRNA ratios respectively) showed a positive effect on sgRNA library coverage: the 47 cells/sgRNA ratio led to the loss of almost 50% of the sgRNAs in the library, whereas the 90 cells/sgRNA proved to be adequate to cover the full complexity of the library.
- Increasing the number of cells/10X reaction from 2,000 to 7,500 and 15,000 did not adversely affect the magnitude of the observed effect of TNFRSF1A, IKBKG, RELA, CHUK KO on TNFα mediated transcriptional activation
- Based on the results obtained with the small sgRNA library used in this work, we can conclude:

1. To cover the full complexity of a pooled sgRNA library, minimum coverage of ~100 cells/sgRNA should be used.

2. With a loading limit of 15,000 cells/10X reaction, a minimum of one 10X reaction will be needed for every 150 sgRNAs in the library (Chromium Controller, with the new Chromium X in the loading limits should be higher).

3. Irrespective of the Chromium instrument used, the NGS sequencing depth required to analyze the results of the Perturb-Seq experiment will be directly tied to the complexity of the library (each sgRNA in the library will need a full transcriptome-wide RNA seq coverage).

References:
- Dixit, A., Parnas, O., Li, B., Chen, J., Fulco, C., & Jerby-Arnon, L. et al. (2016). Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. Cell, 167(7), 1853-1866.e17.
- Adamson, B., Norman, T., Jost, M., Cho, M., Nuñez, J., & Chen, Y. et al. (2016). A Multiplexed Single-Cell CRISPR Screening Platform Enables Systematic Dissection of the Unfolded Protein Response. Cell, 167(7), 1867-1882.e21. doi: 10.1016/j.cell.2016.11.048 doi: 10.1016/j.cell.2016.11.038