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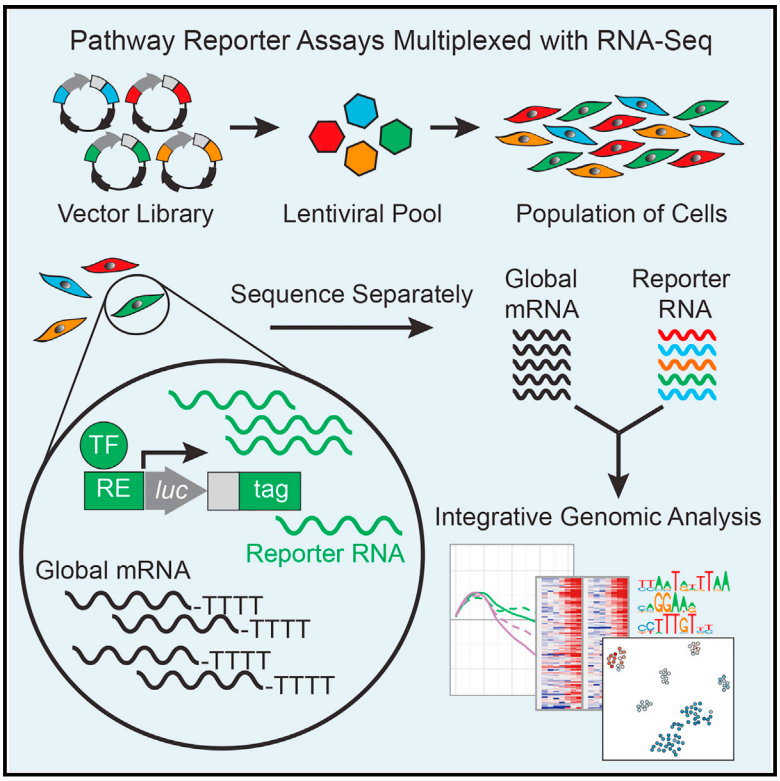
Cell Systems

Simultaneous Pathway Activity Inference and Gene Expression Analysis Using RNA Sequencing

Graphical Abstract



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In Brief

O'Connell et al. use RNA-seq to multiplex reporter gene assays and infer the activity of more than 40 signaling pathways in parallel and in hundreds of samples, enabling discovery-oriented applications. Integration with global gene expression profiling from the same cells facilitates a systems-level view of transcriptional response by associating major gene expression features with a small number of transcription factors.

Highlights

- We develop TF-seq to multiplex pathway-specific reporter gene assays using RNA-seq
- TF-seq pathway activity inferences cannot be detected with gene expression data alone
- TF-seq and global mRNA expression profiling can be performed on the same cells
- Integrative analysis associates transcription factors with direct target genes

Accession Numbers

GSE75212

O'Connell et al., 2016, Cell Systems 2, 323–334
May 25, 2016
http://dx.doi.org/10.1016/j.cels.2016.04.011

CellPress

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Simultaneous Pathway Activity Inference and Gene Expression Analysis Using RNA Sequencing

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SUMMARY

Reporter gene assays are a venerable tool for studying signaling pathways, but they lack the throughput and complexity necessary to contribute to a systems-level understanding of endogenous signaling networks. We present a parallel reporter assay, transcription factor activity sequencing (TF-seq), built on synthetic DNA enhancer elements, which enables parallel measurements in primary cells of the transcriptome and transcription factor activity from more than 40 signaling pathways. Using TF-seq in *Myd88*$^{-/-}$ macrophages, we captured dynamic pathway activity changes underpinning the global transcriptional changes of the innate immune response. We also applied TF-seq to investigate small molecule mechanisms of action and find a role for NF-κB activation and coordination of the STAT1 response in the macrophage reaction to the anti-inflammatory natural product halofuginone. Simultaneous TF-seq and global gene expression profiling represent an integrative approach for gaining mechanistic insight into pathway activity and transcriptional changes that result from genetic and small molecule perturbations.

INTRODUCTION

Cellular signaling networks integrate external and internal information through biochemical interactions that ultimately regulate transcriptional responses. Reporter assays provide a quantitative assessment of signal transduction pathway activation by inferring the activity of pathway-specific transcription factors in terms of protein activity (Gorman et al., 1982; Bronstein et al., 1994). However, this approach is not amenable to the analysis of multiple signaling pathways in a single population of cells due to a paucity of orthogonal protein activity readouts (Bellis et al., 2011; Padmashali et al., 2014). In contrast, global gene expression profiling offers a more information-rich, unbiased approach to identify key mediators of signal transduction and gene regulation (Tian et al., 2005). Yet, it is not straightforward to reliably reconstruct upstream signaling events by means of gene expression data alone. The use of RNA sequencing (RNA-seq) in gene reporter assays has been described successfully in enhancer studies (Arnold et al., 2013; Melnikov et al., 2012; Patwardhan et al., 2012), analysis of mRNA structure and function (Holmqvist et al., 2013; Zhao et al., 2014), examinations of chromosomal position effects (Akhtar et al., 2013), and interpretation of noncoding genetic variation (Vockley et al., 2015). However, a high-throughput sequencing (HTS) approach to systematically isolate and assay the activity of multiple distinct transcriptional regulators in parallel has not yet emerged.

To address these limitations, we developed an HTS assay termed transcription factor activity sequencing (TF-seq), and we demonstrate its application to canonical transcription factors from more than 40 widely investigated signaling pathways. Because TF-seq is based on RNA-seq technology, we were able to simultaneously integrate global gene expression and signaling pathway activity measurements. TF-seq produced inferences of pathway activation in primary macrophages after stimulation with a diverse panel of microbial stimuli; these inferences could not be recapitulated by RNA-seq or chromatin immunoprecipitation sequencing (ChIP-seq) data alone. We then used TF-seq to investigate the mechanism of action of the anti-inflammatory natural product halofuginone (HF) in primary macrophages, identifying an unexpected activation of NF-κB and suppression of STAT1.

RESULTS

TF-Seq Quantitative Methodology and Experimental Design

TF-seq is based on 58 lentiviral reporter vectors that are distinguished only by the unique DNA response element (RE) cloned in front of the luciferase (Luc2P) open reading frame and its