Washington University in St. Louis
Office of Technology Management

SINGLE CELL CALLING CARDS (SCCC): TRANSPOSON SYSTEM TO SIMULTANEOUSLY MAP TRANSCRIPTION FACTORS AND QUANTIFY GENE EXPRESSION

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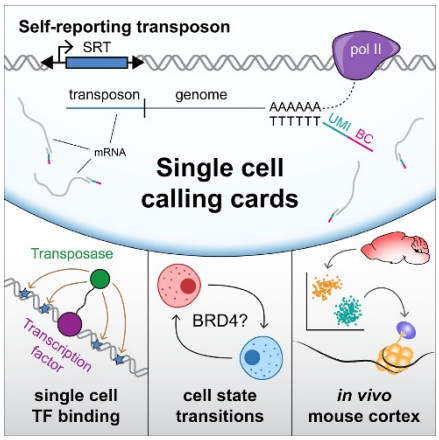
T-018609

Technology Description

Researchers in Prof. Rob Mitra's laboratory have developed single cell calling cards ("scCCs"), a robust and versatile self-reporting transposon system for high throughput analysis of protein-DNA binding with single cell resolution. This technology simultaneously maps the precise location of transcription factor (TF) binding sites and measures the corresponding gene expression using scRNA-seq techniques that are compatible with standard droplet microfluidic platforms.



Overview of scCC system and proof of concept demonstrations

The scCC assay adds a self-reporting feature to previous TF-transposase "calling card" tools developed in the Mitra lab. This new system inserts a transposon at the location of TF DNA binding events and drives transcription at that site through a strong promoter. Importantly, the transcript is not terminated in the transposon but instead continues into the neighboring genomic DNA, enabling the transposon location to be read out in cellular RNA by single cell sequencing. This read out is then used to link the TF binding site to the identity of the cell. scCC can be used to map transposon locations from thousands of single cells in parallel for genome-wide identification of TF binding sites across multiple cell types in complex and heterogeneous tissue. End user applications of this technology include cell lineage analysis, transposon mutagenesis screens and basic research to elucidate TF biology.

Stage of Research

The inventors have created novel scCC constructs with a variety of transposon systems and demonstrated them both *ex vivo* and *in vivo* by benchmarking multiple kinds of DNA binding proteins, including sequence-specific TFs (SP1 and FOXA2) and indirect chromatin-associated factors (BRD4 and BAP1):

- *Ex vivo* – mapped multiple transcription factors in several cell lines; discovered bromodomain dependent cell-state transitions in leukemic cells (K563)
- *In vivo* – mapped and characterized multiple transcription factors in heterogeneous tissue (mouse cortex)

Publications: Moudgil, A., Wilkinson, M. N., Chen, X., He, J., Cammack, A. J., Vasek, M. J., ... & Morris, S. A. (2020). Self-reporting transposons enable simultaneous readout of gene expression and transcription factor binding in single cells. *Cell* (also available on bioRchiv).

Applications

- **Transcription factor/DNA-protein interaction profiling** with end user applications such as:
  - basic research of TF biology and transcriptional regulation of dynamic systems
  - cell fate mapping using transposon insertion as barcodes of developmental lineage
  - massively multiplexed transposon mutagenesis screens

Key Advantages:

- **Single cell resolution** from simultaneous read-out of mRNA content and transcription factor (TF) binding event
  - directly and reliably maps site of TF and quantifies the abundance of the mRNA transcript at that location
  - genome-wide analysis not restricted to predetermined cell types
  - scCC method allows cell-type-specific mapping of SRTs from scRNA-seq libraries
- **Robust and versatile assay**:
  - general technique can be used in a variety of cell lines with multiple TFs and transposon systems (e.g., piggyBAC, Sleeping Beauty, L1 retrotransposon, Helitron)
  - tracks TF binding in a variety of challenging ex vivo and in situ models, including heterogenous tissue
- **High throughput analysis**:
  - maps locations of self-reporting transposons from thousands of single cells in parallel with scRNA-seq
  - compatible with standard droplet microfluidic platforms
  - potential for multiplex studies of TF binding

Patents: Compositions of self-reporting transposon (srt) constructs and methods for mapping transposon insertions (U.S. Patent Application, Publication No. 20200181626)

Related Web Links: Mitra Profile, Mitra Lab