Single-cell mapping of lineage and identity via CellTagging

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ABSTRACT

Single-cell technologies are offering unprecedented insight into complex biology, revealing the behavior of rare cell populations that are typically masked in bulk population analyses. One current limitation of single-cell approaches is that lineage relationships are lost as a result of cell processing, restricting interpretations of the data collected. Elegant computational approaches have been developed in an effort to infer these missing observations, but it remains a challenge to reconstruct true reprogramming trajectories using these tools. Although sophisticated lineage tracing solutions to connect cell history with fate are emerging, these protocols are either not compatible with high-throughput scRNA-seq, or require genome editing strategies that are not readily deployed in some systems. Here, our protocol describes a single-cell resolution clonal tracking approach, 'CellTagging', based on combinatorial cell indexing, permitting the parallel capture of lineage information and cell identity. CellTagging integrates with high-throughput single-cell RNA-sequencing, where iterative rounds of cell labeling enable the construction of multi-level lineage trees. This straightforward lentiviral-labeling approach can be applied to an array of cell biological applications to simultaneously profile lineage and identity, at single-cell resolution.

TAGS

Single Nuclei RNA-Seq

Reprogramming

Show tags

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.biorxiv.org/content/early/2017/04/28/127860

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

MATERIALS

NAME	CATALOG #	VENDOR
Stellar Competent Cells	636763	Takarabio
CellTag pooled library V1	115643	addgene
CellTag pooled library V2	115644	addgene
CellTag pooled library V3	115645	addgene

SAFETY WARNINGS

For generation of lentivirus, the appropiate institutional regualations must be satisfied.

Amplification of CellTag library

1

Thaw Stellar Competent Cells in an ice bath just before use.

2 After thawing, mix 100 uL of cells with 10-50 ng of CellTag V1 DNA in a 1.5-mL microcentrifuge tube.

3 Place transformation mixture on ice for 30 minutes.

4 Heat shock the cells for exactly 60 seconds at 42 °C .

5 Place tube on ice for 1-2 minutes.

6 Add SOC medium to bring the final volume to 1000 uL.

7 Incubate by shaking (~250 rpm) for 1 hour at 37 °C .

8 Take 6 uL of the recovery. Use 1 uL of this to prepare a 1:10 and then a 1:100 dilution. Plate both dilutions and the rest of the 5 uL of recovery onto three separate LB/Amp plates. Grow overnight at 37 °C .

9 Add the rest of the recovery to 500 mL of LB+Amp. Grow overnight while shaking (~250 rpm) at 37 °C .

10 After counting the CFUs from the LB/Amp plates the next day, calculate the transformation efficiency.

11 Harvest the cells from the liquid culture and use multiple Maxiprep columns (Sigma or Zymogen) to purify the library.

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