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Single-cell mapping of lineage and identity via CellTagging V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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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.

Materials

MATERIALS

- Stellar Competent Cells **Takara Bio Inc. Catalog #**636763
- CellTag pooled library V1 **addgene Catalog #**115643
- CellTag pooled library V2 **addgene Catalog #**115644
- CellTag pooled library V3 **addgene Catalog #**115645

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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