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

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We are still developing and optimizing this protocol

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Keywords: cell barcoding, lineage tracing, single-cell analysis, direct lineage reprogramming, cell mapping of lineage, parallel capture of lineage information, cell history with fate, identity via celltagging, lineage information, cell mapping, cell rna, cell resolution clonal tracking approach, genome editing strategy, cell history, celltagging, iterative rounds of cell labeling, rare cell population, sophisticated lineage, combinatorial cell indexing, unprecedented insight into complex biology, cell labeling, lineage, behavior of rare cell population, lineage relationship, cell identity, rna, cell approach, cell, cell processing, cell resolution, bulk population analysis, reprogramming trajectory, complex biology

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

Troubleshooting

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.

12