Combining Cell Barcoding and CRISPR sgRNA Libraries with Targeted Gene Expression for Single Cell Genetic Analysis of Tumor Metastasis Development

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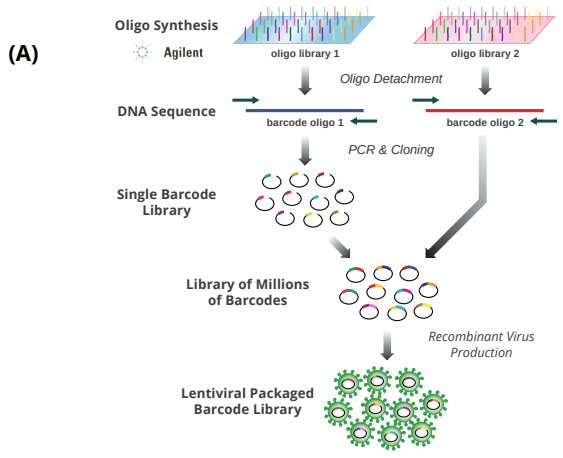
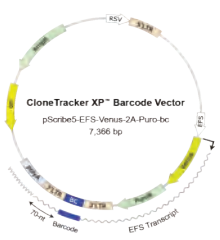
Abstract

Pooled lentiviral libraries of CRISPR sgRNAs to mediate genome-wide gene knockout have become an invaluable tool for uncovering the functional genetic drivers required for a biological response. Another type of pooled lentiviral library designed with unique DNA sequence tags has also been used to label large populations of cells with unique cell-specific barcodes which allows for monitoring changes in sub-populations of cells with distinct phenotypes over time. We have combined cell barcodes with CRISPR sgRNA to construct libraries that enable the identification of multiple occurrences of a common phenotypic response from independent transductions of the same sgRNA effector. Specifically, we demonstrate how this sort of library can be used to identify genes whose activation promotes metastasis of tumors derived from MDA-MB-231 cells engrafted into mice after transduction with a barcoded CRISPR-activation (CRISPRa) sgRNA library. By incorporating multiple barcodes with each sgRNA effector, it is possible to see that, not only are cells in metastatic tumors more likely to have a particular sgRNA targeted to a certain gene, but also that independent transductions of that sgRNA sequence lead to multiple independent metastatic events. With multiple independent clones producing the same phenotype, it is possible to confidently isolate the sgRNA, and by implication, the increased activation of its gene target, as the primary cause of the metastatic phenotype.

Making Complex Lentiviral Barcode Libraries

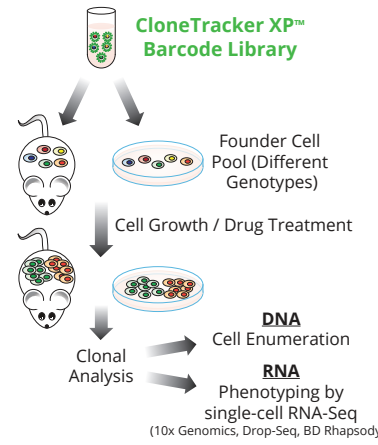




Panel A: Cellecta's approach to make pooled lentiviral libraries with 1000's of oligos can be adapted to produce highly complex lentiviral libraries with several million uniquely identifiable defined barcodes. Barcodes can also be cloned in combination with sgRNA and shRNA to enable individual tracking of each cell that is transduced with the library.

Panel B: Barcodes can be positioned in the vector so that they are expressed, which enables them to be read by both DNA sequencing and RNA sequencing.

Cell Labeling and Clone Tracking with Barcode Libraries

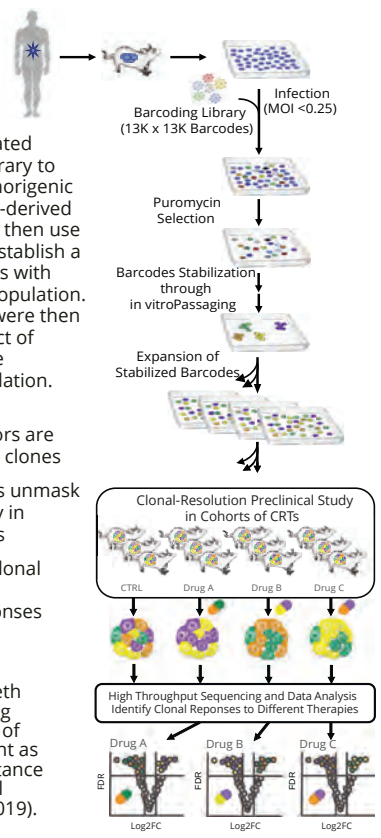


Transduction of complex barcode libraries can be used to label a "founder cell" population where each cell contains an individual barcode. Changes in the clonal diversity and associated sub-population phenotypes of these labeled cells through expansion and selection in a range of *in vivo* and *in vitro* models can be monitored by NGS and, for some libraries, RNA sequencing.

Applications:

- Screening of genes critical for cell growth
- Discovery of synergistic & direct targets for novel drugs
- Tracking gene knockout, activation (CRISPRa), inactivation (CRISPRi)

Analysis of Clonal Tumorigenic Populations

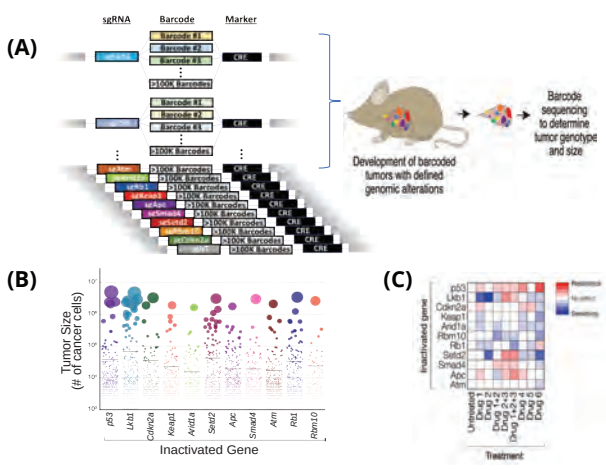


A recent study demonstrated use of a CloneTracker Library to identify and culture a tumorigenic sub-population of patient-derived xenograft (PDX) cells, and then use of these labeled cells to establish a series of xenograft tumors with similar barcoded clonal population. These barcoded models were then used to analyze the impact of various treatments on the tumor-initiating cell population.

- Lineage tracing reveals in vitro and in vivo tumors are maintained by common clones
- Pancreatic clonal tumors unmask functional heterogeneity in response to therapeutics
- Gemcitabine-naïve subclonal gene signature predicts chemotherapeutic responses

Data and Figures from: Seth A., Li C-Y. et al. Pre-existing Functional Heterogeneity of Tumorigenic Compartment as the Origin of Chemoresistance in Pancreatic Tumors. Cell Reports 26: 1518-1532 (2019).

Quantifying Tumors Resulting from *in Vivo* CRISPR Knockout



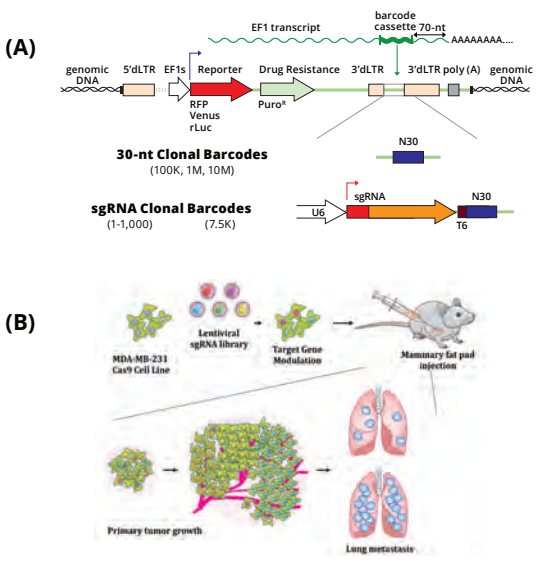
Panel A: Lung tumors were initiated by inhalation of a library of lentiviral constructs, each containing unique clonal barcodes and and sgRNA targeting a tumor-suppressor gene.

Panel B: The relative sizes of tumors resulting from each sgRNA-barcode combination were assessed after 15 weeks using NGS read counts. The graph shows the number of transductions by each barcoded tumor-suppressor construct and the relative sizes of resulting tumors from each transduction.

Panel C: Different drug combinations were incorporated into the mice models described above. The grid shows the change in the level of tumorigenesis for inactivated tumor suppressor genes in the presence of a specific drug treatment.

Data and Images from: Rogers, Z. N., et al., Nature Genetics: 50, 483–486 (2018)

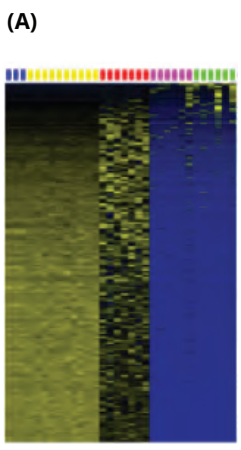
In Vivo CRISPRa Screen to Identify Inducers of Metastasis

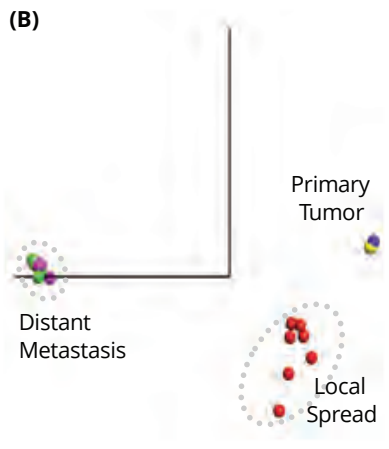


Panel A: A CloneTracker lentiviral library made with CRISPRa sgRNAs (sgRNAs designed to target the promoter region and activate gene expression) targeting key regulators of motility, tumor suppressors, EMT.

Panel B: MDA-MB-231 cells transduced with the CloneTracker barcoded CRISPRa library were injected into mouse fat pad cells leading to the formation of primary tumors. Metastasis of these primary tumors led to secondary tumor formation in lung and other tissues.

Analysis of CRISPRa Screen with CRISPRa Barcode Library





Panel A: NGS analysis of the sgRNA-barcode constructs found in the pre-injected cells, primary tumor, and secondary metastatic tumors. Analysis shows significant enrichment of subset of sgRNAs in the in metastasis samples versus primary tumor and transduced MDA-MB-231 cells.

Panel B: PCA analysis demonstrates the similarity of CRISPRa sgRNAs enriched in the metastatic tumors, as compared to the primary tumor and pre-injected cells.

Conclusions

Lentiviral clonal barcode libraries provide a powerful tool to label and track cell lineages in both *in vitro* and *in vivo* cell systems. Examples in this poster show how these libraries can be used in for three different types of *in vivo* studies:

1. Establish a stable barcoded population of tumorigenic PDX cells that can be used to analyze tumor development, as well as study tumor cell evolution and metastasis in model systems and in response to different treatments.

2. Analyze and quantitatively measure tumor induction and growth in response to CRISPR knockouts to provide a model to assess, in parallel, the effect of various treatments in conjunction with a range of genetic disruptions.

3. Track and identify genetic activations that increase metastatic potential.

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