Lineage-specific Isolation of Barcode-labeled Breast Carcinoma Cell Subpopulations

Authors: Michael Miramontes, Daylin Morgan, Aziz Al'Khafaji and Amy Brock

Introduction: Many current healthcare challenges including cancer and infectious diseases are controlled by the evolutionary dynamics of heterogenous cell populations. In cancer, evidence shows that intratumoral heterogeneity is a contributor to chemoresistance and metastasis driven by rare mutations and epigenetic changes. High-diversity DNA barcode libraries stably integrated into cells have been used to track these populations over time. However, uncovering these lineage dynamics has been a primarily destructive process. Recently, our lab has developed a lineage-tracing platform, Control of Lineage by Barcode Enabled Recombinant Transcription (COLBERT), to precisely monitor heterogenous subpopulations within a tumor. Importantly, this platform also affords us the ability to isolate specific subpopulations through activation of a lineage specific gene expression circuit. Here we demonstrate successful isolation of subpopulations within MDA-MB-231 breast carcinoma cells treated with doxorubicin.

Materials and Methods: A high diversity barcode library of greater than 10^6 unique barcodes was generated and transduced into MDA-MB-231 cells with low MOI. Successfully transduced cells expressing eGFP were isolated using FACS to generate a population with starting diversity of ~1000 unique barcodes. Expressed barcodes were amplified out of the sorted population and assessed using PCR and Illumina sequencing. Barcoded MDA-MB-231 cells were then treated with a high dose of doxorubicin (550 nM, LD_{99}) for 48 hours and allowed to recover, followed by isolation of genomic DNA, barcode amplification and Illumina sequencing. Plasmids with lineage-specific gene expression circuits were generated for abundant post-treatment populations. These recall plasmids were co-transfected with dCas9-VPR in cells from pre-and post-treatment and isolated using FACS.

Results and Discussion: Using a recall plasmid to drive lineage-specific expression of GFP and single cell FACS, a resistant lineage from a pre-treatment sample (BgL1K) and two post-treatment samples (Rel-1, Rel-2) was isolated. This lineage comprised <2% of BgL1K and Rel-1 and ~90% Rel-2. Thus, Rel-2 was single cell sorted without transfection and served as a negative control in the sort. BgL1K and Rel-1 were transfected with the recall plasmid and showed 0.2% and 0.17% GFP activation, respectively. A subset of these clones were successfully expanded in culture. To confirm lineage identity, barcodes were amplified by PCR and sequenced by Sanger sequencing. In total, we obtained 12 clones of BgL1K, 7 clones of Rel-1 and 12 clones of Rel-2 consisting of the lineage of interest.

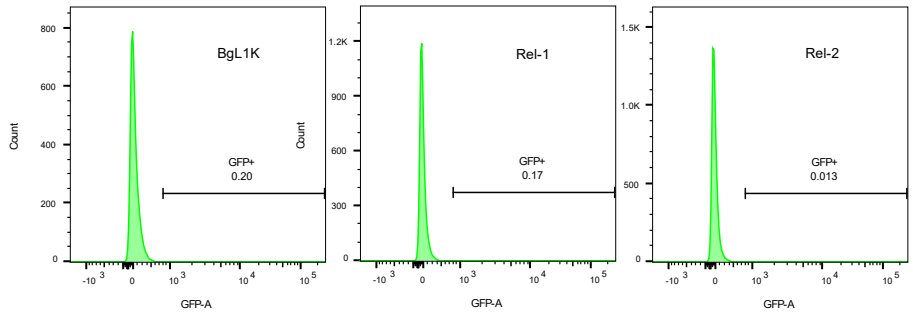

Figure 1: A single lineage was isolated using single cell FACS. Rel-2 was not transfected and served as a negative control. BgL1K and Rel-1 had a GFP activation of 0.2% and 0.17% respectively.

Conclusions: Utilizing COLBERT, we have demonstrated the ability to track lineages of interest in a high-diversity population as well as drive lineage specific gene expression. Here we do so by isolating lineages from pre-and post-treatment with a cytotoxic chemotherapy agent. We are now able to perform growth analysis and screen additional therapeutic agents. With these isolated subpopulations, we are able to determine possible pre-existent states or phenotypic changes that occurred in response to treatment.

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