UCLA Quant & Sys Bio JC PREreview of "Memory sequencing reveals heritable single cell gene expression programs associated with distinct cellular behaviors"

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This is a preprint Journal Club review of **Memory sequencing reveals heritable single cell gene expression programs associated with distinct cellular behaviors** by Sydney M Shaffer, Benjamin L Emert, Ann E. Sizemore, Rohit Gupte, Eduardo Torre, Danielle S Bassett, and Arjun Raj. The preprint was originally posted on July 27, 2018 (DOI: https://doi.org/10.1101/379016).

Dear authors,

Thank you for posting your work as a preprint on BioRxiv. We discussed your work at our latest quantitative and systems biology journal club at UCLA. Below is a summary of our feedback containing our main remarks, points of discussion, and suggestions.

This study aimed to identify genes and groups of genes that exhibit memory persisting over multiple cell divisions. The authors hypothesized that any such genes would manifest phenotypically as rare cell subsets within a seemingly homogeneous population. To test their hypothesis, they developed a clever new method, termed MemorySeq, which adapts the classic Luria-Delbrück fluctuation experiment to examine gene expression at the genome scale. In this experiment, a clonal population of cells was passed through a bottleneck and gene expression was quantified by RNA sequencing. Genes that exhibited high inter-clonal expression variability were identified as exhibiting multi-generational memory. MemorySeq also allowed them to identify genes that conferred drug resistance, confirming that heritable expression can create specialized cell subsets. In general, the methods were innovative and well-suited to analyze gene expression heritability at the systems scale. The paper was very clearly written and the figures were, for the most part, well-presented. Our comments are outlined below.

Major points

To validate MemorySeq results at the single cell level, the authors used single molecule RNA FISH and time-lapse fluorescence microscopy. Time-lapse microscopy was used to establish a rough estimate for the fluctuation timescale (for NGFR) and conclude that cells traverse the high and low states over an 8 day period. Given the limited scope of data presented, it is difficult to know if this is true. Considering experimental variability and measurement error, we do not believe that a single cell (Fig. 2E) is sufficient to establish this conclusion. A larger sample of cells exhibiting this behavior, along with some quantification of the fluctuation timescales, would help to make the argument more convincing. In addition, a comparison should be made to a fast fluctuating (non-heritable) gene as a control. Second, the images are difficult to interpret as they seem to contain imaging or analysis artifacts (such as lines and saturated brightness). This should be corrected. Third, we found it surprising that the authors did not take advantage of the power of this method to further

corroborate MemorySeq. Although we appreciate the significant challenges associated with tracking cells over 8 days of imaging (especially given the motility of certain cancer cell lines), a lineage analysis would provide excellent validation for their LD experiment. One approach to improve the performance of automated tracking is to plate H2B-iRFP/NGFR-mNeon green cells sparsely amongst unmarked cells and track only the marked cells.

Next, the authors presented a method by which new resistance genes might be identified. They validate the method by identifying two genes (NGFR and CA9, Figs. 3 and 4) that have been previously linked to resistance. The authors should reference previous work identifying and mechanistically characterizing the relationship between CA9 expression and drug resistance[1-3]. Demonstrating the potential power afforded by MemorySeq in finding a novel target(s) would elevate the impact of the work. After identifying such a target, the authors could sort based on the expression of that gene and perform resistance assays.

The authors attempt to link drug resistance to heritable gene expression. However, they don't directly test the hypothesis that *heritable* gene expression confers a selective advantage to drug treatment. We think the best approach would have been a classic Luria-Delbrück experiment: treat different clonal lineages separately with drugs and measure survival. One would expect a greater degree of inter-clonal variability in survival compared to that of mixed populations (similar to noise control). This could be achieved by expanding single clones and treating each population with drug (where cells are guaranteed to be related) and comparing the fraction killed to a mixed noise control. If the authors' conclusion is true, this experiment should reveal that most wells are entirely susceptible to drug while a small percentage are largely resistant, giving the distinctive long-tail distribution observed for gene expression of resistance-related genes.

Minor points

We were curious about what exactly the heritability genes were. Aside from the obvious clinical relevance of resistance genes, we were excited to know what the ontogeny signatures would reveal about heritability genes. In other words, what genes are cells designed to inherit best? Although the LD genes from the two cell lines were not highly overlapping, it would have been interesting to see whether they possess overlapping GO signatures.

Regarding the analysis of co-regulated genes (Fig. 5C), we felt that the comparison of R^2 values between the bulk RNA-Seq and smFISH would have been better visualized and interpreted using a scatter plot instead of heatmaps.

When discussing "intermediate memory", paragraph 2 of main text. The authors should mention some of the papers that take genetically identical cells and investigate population heterogeneity maintained across divisions due to inherited gene expression of molecular network state[4-8].

Thank you again for posting your work on BioRxiv. We had a wonderful discussion about the paper in our journal club and we hope you find our comments useful.

Author names (ordered alphabetically)

Alon Oyler-Yaniv, Jen Oyler-Yaniv, Maeve Nagle, Ryan Lannan, Simon Mitchell, Zachary Hemminger, and all of the remaining members of the WESQWorld Journal Club

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