The new Teton™ chemistry on AVITI enables high content cellular profiling and cell morphology analysis

Daniel Honigfort[1], Mariam Dawood[1], Connor Thompson[1], Ramreddy Tippana[1], Sanchari Saha[1], Tyler Lopez[1], Zachary Burns[1], Pin Ren[1], Jennifer Wong[1], Da Zhao[1], Jerry Wu[1], Colin Brown-Greaves[1], Jean Kwon[1], Yu Liu[1], Adeline Huizhen Mah[1], Derek Fuller[1], Ryan Kelley[1], **Sinan Arslan**[1], Michael Previte[1]

[1]Element Biosciences, San Diego, CA

Element Biosciences

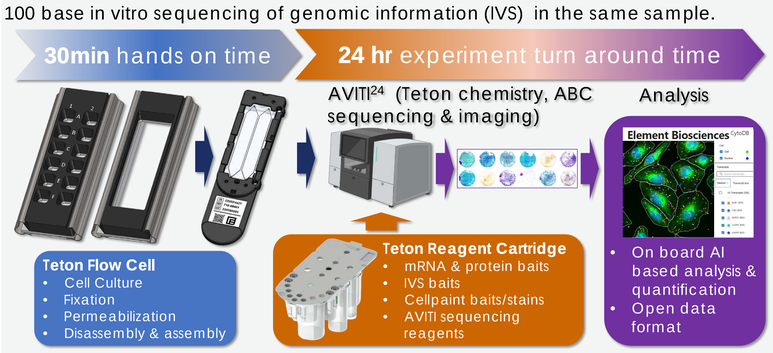
Introduction

Currently, researchers who need sensitive, reliable information about RNA/protein expression, localization, and cell morphology at the subcellular level need to use multiple assays and often multiple instruments. Here, we present an advancement in the Element Biosciences AVITI™ sequencing platform that allows researchers to collapse multiomics data collection into a single assay using **Avidity Based Chemistry (ABC)**.

With a flexible 30-minute hands-on time workflow, the AVITI[24]™ platform can concurrently assess RNA and protein expression levels in situ at high plexity, capture detailed morphology data, and sequence transcriptional information in up to millions of cells per flow cell (FC) in an area up to 20 cm^2. These assays are performed using a standard commercial AVITI system and cartridge with the addition of proprietary Element ABC and **Teton™** chemistries. With this new functionality, the AVITI[24] system can return fully integrated multiomic data with one box, in one experiment, in less than one day.

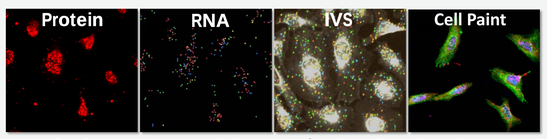
Methods

AVITI[24] simultaneously allows high plexity detection of protein and mRNA levels, no crosstalk/scalable cell paint for morphological analysis, with the option of longer than 100 base in vitro sequencing of genomic information (IVS) in the same sample.



To achieve this, cells are seeded and cultured (treated as described in Results) on single-well or 12-well Teton FC and. Our proprietary surface chemistry supports cultures of a variety of cell types. ABC in cells is also compatible with other common surface treatments e.g., polylysine or collagen. After fixation and permeabilization, FCs are assembled into the AVITI[24] FC.

AVITI[24] reagent cartridge contains Element proprietary high plex chemistry using ABC and Teton chemistries for specific mRNA and protein panels, cell paint targets, and in vitro sequencing, as well as reagents for internal controls and calibrations. The AVITI[24] imaging system captures the ABC sequencing information with subcellular resolution in a wide field and fast turn around time. AVITI[24] performs cell segmentation as well as transcript and protein quantification on board during the run, and outputs data for cloud and third-party tools.

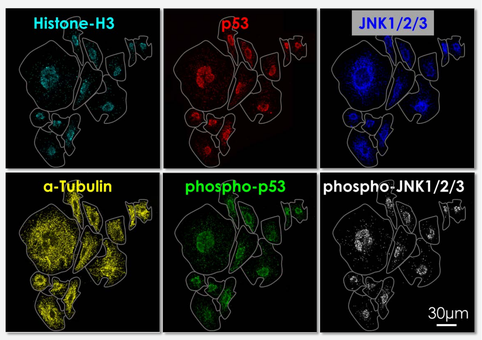


mRNA and protein data generated by AVITI[24] chemistry has been validated against FISH, sc/bulk RNAseq, IF, and WB. We are also introducing new metrics to evaluate the sensitivity and specificity of our detection.

Results

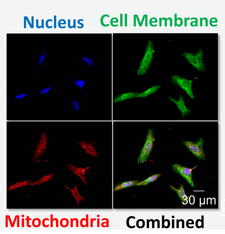
Protein detection

Six proteins were visualized with subcellular resolution on AVITI[24] using probe chemistry and ABC sequencing signal. Histone-H3, p53, phospho-p53, and phospho-JNK1/2/3 are anticipated to be localized within the cell's nuclear region, while JNK1/2/3 and α-tubulin are expected to be present in the cytoplasm. Moreover, the treatment with TNFa resulted in the upregulation of phospho-proteins.



Cell paint

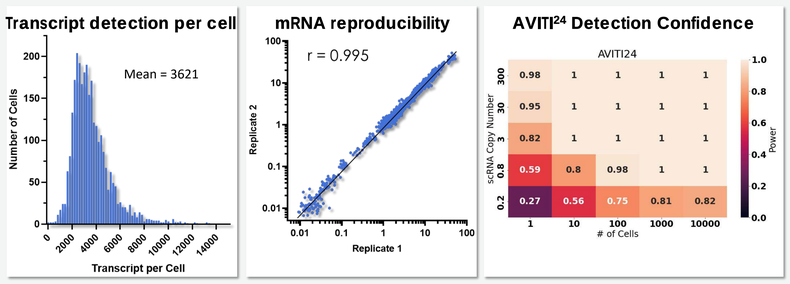
AVITI[24] chemistry enables modular cell paint profiling alongside RNA and protein detection, with no additional hands-on time. Cell paint targets exhibit no cross-talk due to Teton chemistry design, enabling high information content morphological profiling through multiplexing of up to 20 possible morphology targets.



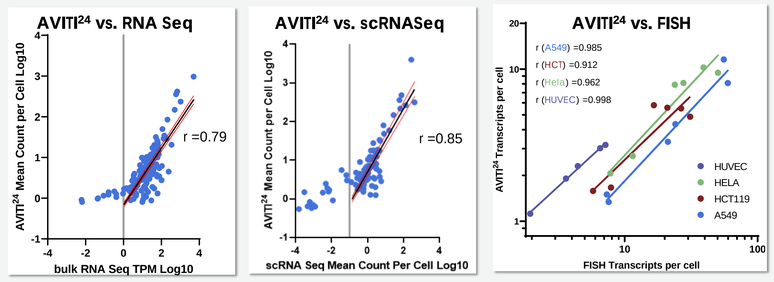
Results

RNA detection

261 plex mRNA panel detection yields 3,621 mean transcript counts per Hela cell, with robust correlation between replicate experiments (r = 0.995). With an achievable cell density of >50,000 cells per well, confidence that detected transcripts are above background is high, down to at least 0.2 copies per cell.

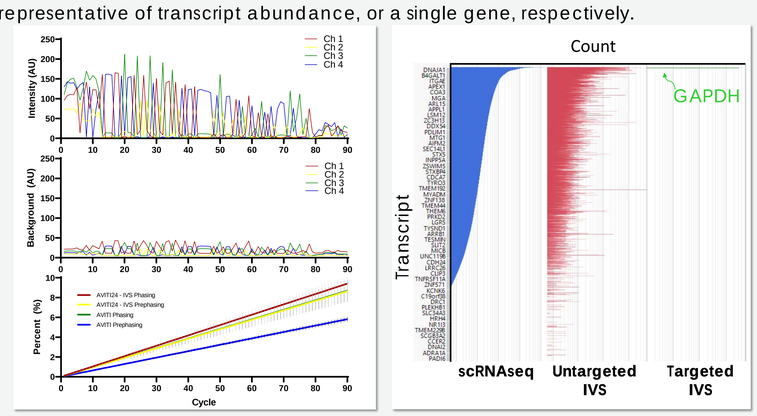


Additionally, mRNA transcript counts on AVITI[24] correlated well with single cell and bulk RNAseq , as well as with fluorescent in situ hybridization (FISH).



Library prep free in vitro ABC sequencing in cells (IVS): 100 bp in < 20 hours

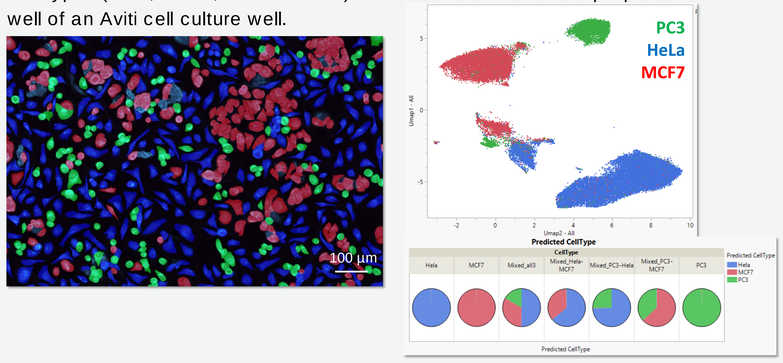
Direct sequencing of RNA in cells was performed for untargeted (total transcriptome) or targeted (specific to a single gene) IVS. Subsequent alignment produced a distribution representative of transcript abundance, or a single gene, respectively.



Low concentration avidity reagents in ABC cycling chemistry produce very little background signal in cells and very low phasing/prephasing. This enables high quality sequencing of transcriptional information in cells up to 100bp.

Multicell - model system

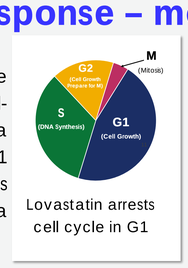
To test the ability of AVITI[24] to distinguish between different cell populations, three cell types (Hela, MCF7, and HCT116) were seeded in different proportions in each well of an Aviti cell culture well.



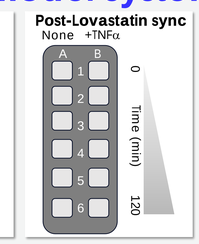
Utilizing mRNA, protein, and morphological information, AVITI[24] readout enables clustering of the three cell types in a single well, and cell type prediction via supervised learning. Confirming the effectiveness of AVITI[24] cell profiling, in wells with a subset of the three cell types present, there are less than 0.2% false positive detections of absent cell types.

Time-dependent cell response – model system

Next, we utilized AVITI[24] to monitor the time dependent response of cells to two well-characterized external stimuli. Lovastatin is a synchronization agent that arrests cells in G1 phase, while Tumor Necrosis Factor (TNFα) is a pro-inflammatory cytokine with roles in a variety of cellular processes.



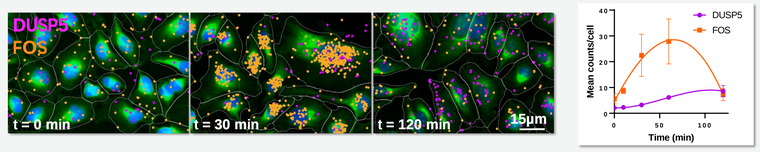
Lovastatin arrests cell cycle in G1



Here we sought to determine the impact of TNFα stimulation on cells previously synchronized in G1 by Lovastatin. After cell cycle arrest overnight, cells were released with the removal of Lovastatin from culture media at time t = 0 min, with half of wells concurrently treated with TNFα. At various timepoints, wells were fixed to preserve temporal information, and AVITI[24] was used to visualize multiomic cellular response to both stimuli over time.

Cellular response to release from G1 Synchronization

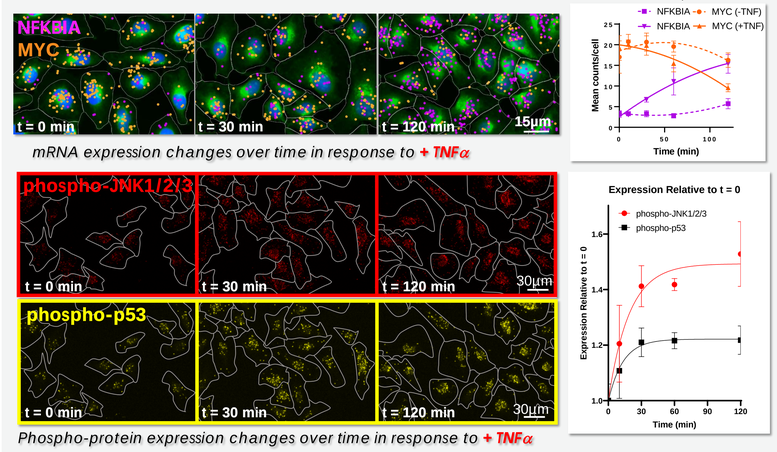
After removal of Lovastatin, cells that were arrested in G1 begin to reenter the cell cycle. Here, we characterized expression changes that are representative of that process in the absence of TNFα to establish a baseline level of regulation.



Cell cycle-specific transcriptional changes observed after release from G1 (- TNFα)

Cellular response to TNFα after G1 Synchronization

Cells that were simultaneously released from Lovastatin and subjected to TNFα treatment exhibit changes associated both with inflammation response and cell cycle reentry. By comparing expression levels between equivalent -/+TNFα timepoints, we observe relative changes due to TNFα in RNA and protein levels.

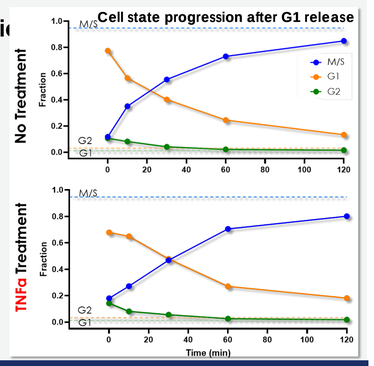


mRNA expression changes over time in response to + TNFα

Phospho-protein expression changes over time in response to + TNFα

Impact of TNFα on cell cycle progressi

Finally, using morphological data from AVITI[24] we can infer the cell state of each cell. While our "unsynchronized" cell population has a low fraction of G1 cells, cells at t=0 post-Lovastatin treatment are predominantly G1 as expected. As time progresses post-release, cells in both -/+ TNFα recovery groups trend toward the S phase. Interestingly, the +TNFα treatment exhibits a slowed rate of exit from G1 phase relative to the non treated cells.



Conclusions

AVITI[24] is an innovative, fully integrated system offering a comprehensive solution for researchers in the fields of RNA and protein analysis, as well as morphological studies and in vitro sequencing in cells. Our results showed 95% concordance with traditional methods down to single digit transcripts and a dynamic range of almost 1000x, while saving weeks and in some cases months on personnel time with a single sequencing run. We aim to develop and expand additional panels cell signaling, immunology, oncology, neurology and liver. AVITI[24] is poised to significantly impact the landscape of molecular and cellular biology and more generally systems biology, offering researchers a powerful tool to unravel the complexities of cellular processes with unprecedented precision and efficiency.