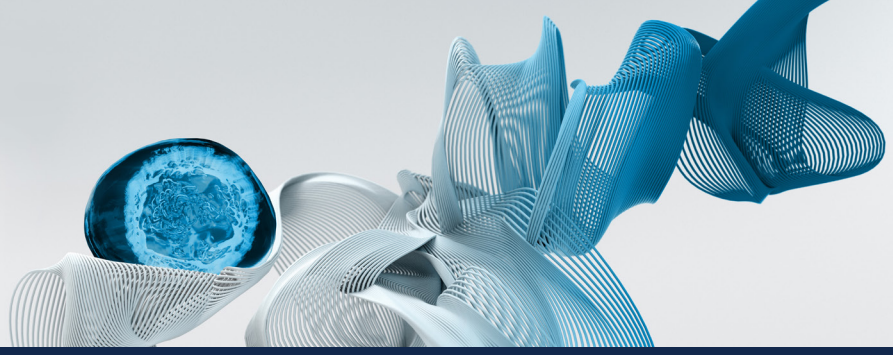
BioSkryb GENOMICS



Product Note

Single-cell Isolation and Enrichment

Cells explored. Answers revealed.

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New Product Introduction
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Open Platforms for Cell Isolation and Enrichment Enabling Downstream Omic Analysis of Specific Single Cells

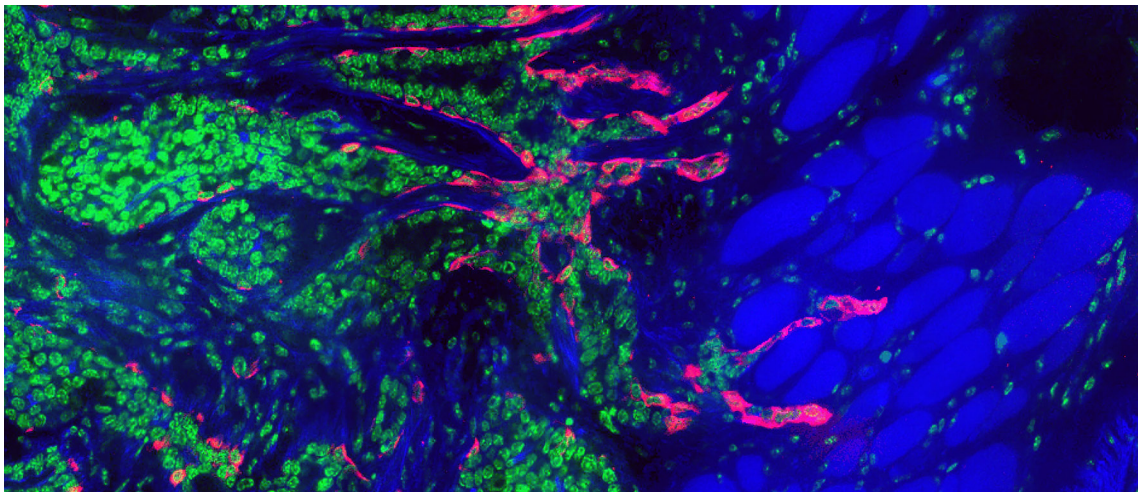


Figure 1 Cellular Heterogeneity drives metastatic invasion of breast tumor cells. In this model, cells which are invasive (pink) can be seen an the luminal edge of the localized ductal tumor (Green cells). https://www.biointeractive.org/sites/default/files/Cancer-Cell.jpg[1]

BioSkryb Genomics has developed a variety of integrated single-cell workflows to enable cell isolation and enrichment. The dissection of cellular heterogeneity depends on the ability to differentiate the omic layers that define an individual cell. Single cell tissue heterogeneity drives cellular evolution in both development and disease (Figure 1). In this case tumor invasion is dictated by multiple effectors within the genome, transcriptome and expressed proteins. Applications and methods developed and reported here utilize several different systems and platforms which enable a wide range of cell acquisition techniques that are compatible with downstream single cell omic analysis. Downstream omic analysis supported by BioSkryb Genomics application systems, include whole genome and whole mRNA transcriptome sequencing, as well as targeted surface protein analysis. In addition, the open platforms allow the user to develop and make use of a wide array of published chemistry platforms. We summarize here the various methods, all having individual strengths and shortcomings.

Cell Isolation and Enrichment

Platforms utilized upstream of omic analysis

- Sony SH800 FACS Enrichment
- NanoCellect WOLF FACS enrichment
- ALS Jena - CellCelector

Downstream chemistry workflows

- ResolveDNA WGA - Single Cell Genome
- ResolveRNA RNAseq - Single Cell Transcriptome
- ResolveOME - Single Cell Multiomics
  - Genome (WGS, WES, Panel)
  - Transcriptome (Full-length mRNA transcript)
  - Integratable with surface marker proteins*
- User-defined single cell chemistries

BioSkryb BaseJumper compatibilities

- Cloud-based Omic data intrepration
- Risk-based analysis using ClinVar/Cosmic
- Synonymous/non-synonymous changes

* Indicate methods in development at BioSkryb Genomics

Introduction

Cellular Heterogeneity drives normal development and disease (Figure 1), through a myriad of intracellular macromolecular factors[1]. At the foundation of these omic layers is the genome of each individual cell. The clonality of cell populations and their phenotypic behavior are influenced, if not dictated by, variation in the genomic structure. Cellular behavior is further influenced by extracellular effectors that may influence gene expression. Our understanding of the complexities of the molecular biology that influence normal development and disease remain immature. The recent broad expansion of both single cell molecular biology approaches, in addition to next generation sequencing (NGS) technologies are allowing a new era of discovery. However, to enable the analysis of each cell or clone, cells must be isolated as individuals. While several single cell platforms have enabled single cell transcriptomics[2], and panel based genetic analysis[3], the systems are inherently limited to the commercial applications provided by the supplier. Given the massive expansion in methodologies, many users seek open systems to implement customized molecular biology methods. The use of open isolation and enrichment platforms that provide application flexibility are beneficial to the single-cell user base.

In addition, while higher throughput experiments are highly valuable for discerning differences between phenotypically variable populations, disease states are most frequently driven by clones of low abundance. Often the specific populations of target cells wishing to be studied are either in low percentage or the total number of cells in the specimen, as with clinical samples, are discrete and limited. Such analyses are not compatible with most droplet or microfluidic based systems. The reality is all systems have their strengths and shortcomings.

At Bioskryb Genomics we differentiate by developing the most comprehensive modal analysis to enable broad and accurate product solutions to determine the structure and function driving the biology of each cell. We have further integrated these chemistry systems with upstream cell isolation and enrichment systems (Figure 2) to maximize flexibility for the unique questions posed by researchers and clinicians. These

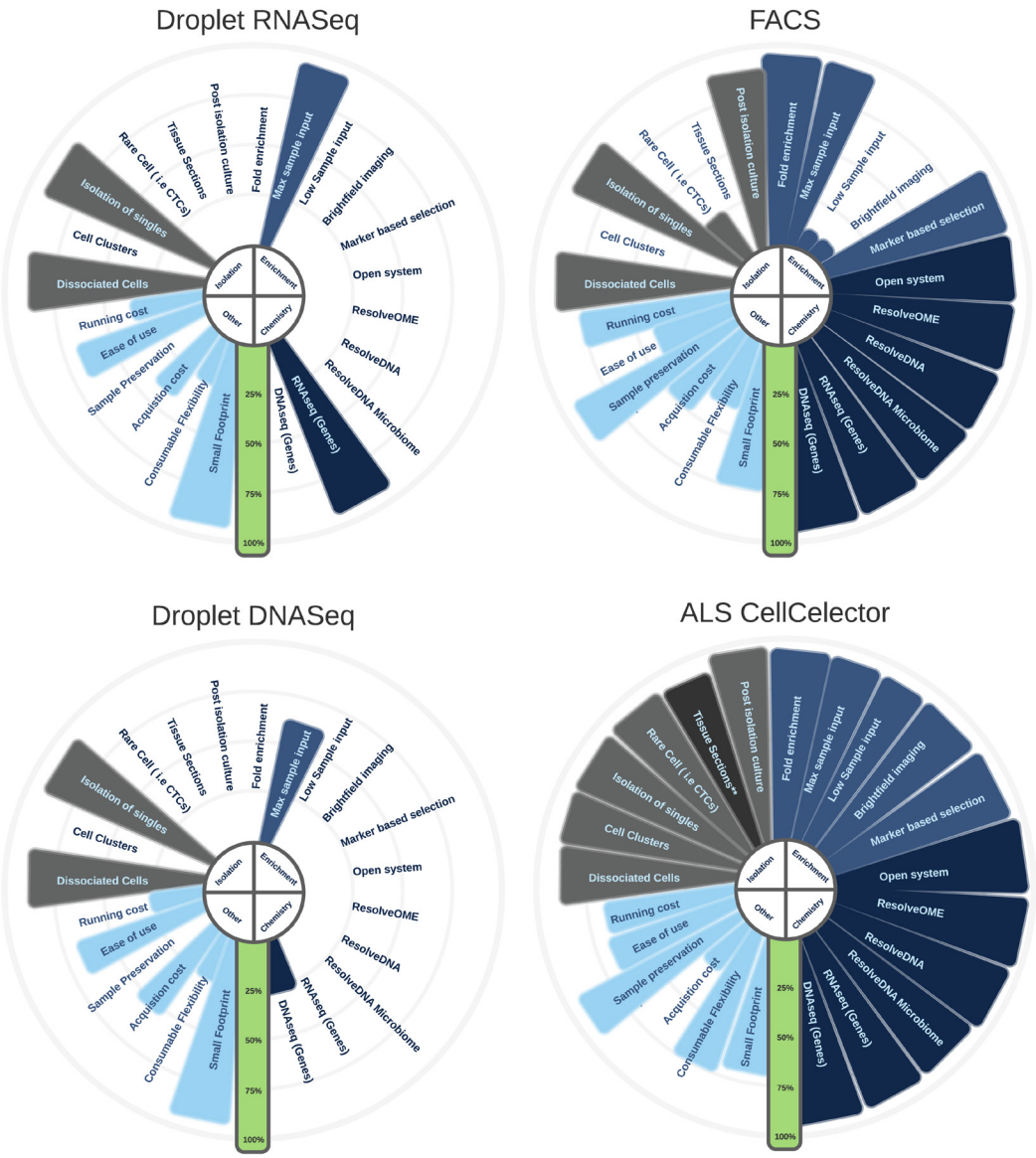


Figure 2. Integration of single-cell methods to cell isolation and enrichment platforms. Existing commercial droplet systems enable high throughput limited parameter single cell analyses. In contrast, using alternative isolation and enrichment systems, such as FACS or the CellCelector, a deeper understanding of the molecular drivers can be accessed for a set of target single cells of interest. These open methods allow significant user flexibility for a broad array of experiment types and questions.

open single-cell platforms (Figure 2) enable significantly greater breadth and depth in cellular and molecular analysis. Compared to the droplet based systems, which have higher cell throughput with limited dimensional analysis (i.e. 3' end counting or a small gene panel), the open systems provide higher throughput in terms of enrichment and allow the user to focus on a target group of cells with greater breadth and depth within the genome, transcriptome and protein analysis. A key feature of BioSkryb Genomics workflows is the application independence we support.

Platform integration

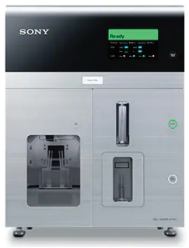
Fluorescent Activated Cell Sorting

Initial PTA-based WGA methods were developed in combination with Fluorescent Activated Cell Sorting (FACS)[4]. Where cell enrichment is a critical experimental requirement, we have developed workflows for both the Sony SH800[5] and NanoCellect WOLF[6] cell sorting systems (Figure 3). Certain applications, such as the analysis of Minimal Residual Disease (MRD) in cancer require significant cell enrichment. In some cases this requires isolating as few as 1 target cell/1 million non-target cells (1x10^6 cells)[7]. Both systems enable index sorting for quantitative assessment of fluorescent gating at the individual cell level. The WOLF sorter has greater ease of use. In contrast, the SH800 provides greater flexibility in fluorescent markers for surface protein detection. Both instruments are compatible with BioSkryb Genomics products and open source chemistry systems.

Spatial Based Cell Selection

Perhaps the most capable of systems is the ALS Jena CellCelector. The CellCelector was designed for single cell studies (Figure 3). Through a combination of high-resolution optics and precise robotics, the instrument has a wide range of capability in the single-cell application sector. Due to the ability to interrogate cells in a sample specimen optically using the array of available consumables, the combination of the CellCelector with the BioSkryb Genomics omic platforms allows the delineation of a broad array of molecular biology layers.

Isolation/Enrichment System	Compatible Consumbles	Application notes


Sony SH 800 FACS
	
	Sony SH800 sorting chips and reagents
		• Live/Dead Sorting
		• Index (enrichment) sorting
		• 6 fluorescent channels
		• Protein surface markers
		• Sorts to 96 & 384 well plates
		• 1:1*10^6 enrichment
		• Minumum input ~50K cells
		• Compatible with
		  • ResolveDNA
		  • ResolveOME
		  • ResolveDNA MicroBiome
		  • Open methods


NanoCellect WOLF FACS
	
	WOLF sorting chips and reagents
		• Low pressure/gentle sorting
		• Live/Dead Sorting
		• Index (enrichment) sorting
		• 2 fluorescent channels
		• Protein surface markers
		• Sorts to 96 & 384 well plates
		• 1:1*10^6 enrichment
		• Minumum input ~50K cells
		• Compatible with
		  • ResolveDNA
		  • ResolveOME
		  • ResolveDNA MicroBiome
		  • Open methods

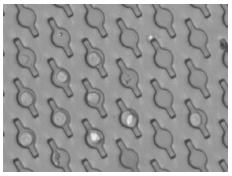
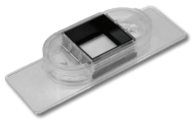

ALS JENA CellCelector
	
	 
	 
		• Live/Dead cell selection
		• Index enrichment selection
		• 6 fluorescent channels
		• Protein surface markers
		• Sorts to well plates:
		  • 96, 384, 1536
		• 1:1*10^5 enrichment (nanoarray)
		• Minumum input ~100K cells
		• Magnetic beads Cell Selection
		• Stem cell isolation/culture
		• Spatial cell selection
		• Compatible with
		  • ResolveDNA
		  • ResolveOME
		  • ResolveDNA MicroBiome
		  • Open methods

Figure 3. Single Cell isolation and enrichment integration with BioSkryb Genomics chemistry systems. Various system integrations allow a range of experimental resolution. The highest enrichment can be accomplished with FACS integration using either the Sony SH800 or NanoCellect WOLF. In contrast, the highest flexibility and accuracy in single-cell experimentation is achieved with the CellCelector.

The CellCelector allows a high degree of population enrichment and a broad range of cell inputs, from just a few cells (< 100) to hundreds of thousands of inputed cells. It accomplishes this through the use of magnetic or optical enrichment or a combination of both. The user is then able to detect and differentiate the cells of interest, and select only those most important for downstream BioSkryb genomic, transcriptomic or protein analysis via NGS. Moreover, we are currently developing methods to enable the isolation and enrichment of specific cells from intact tissue sections. This enables spatial context and precision genomics within the context of an organized tissue sample specimen. Similar to the other platforms described, cells enriched by the CellCelector can be used in a myriad of downstream open source single-cell molecular biology and cloning methods.

Conclusions

The single cell field has advanced with breathtaking pace. In particular, the expansion of high-throughput droplet-based RNAseq methods have elucidated the amazing diversity within tissues. However, the lens we use to determine this diversity is currently limited to end fragments of genes that are expressed or to a handful of genomic targets. In order to understand the complexity of biological function, a new approach is needed. The emergence of multi-parameter single-cell analysis is a step to solving this amazing complexity. As with the diversity of cells, our methodologies for understanding the molecular basis of cellular function must evolve. The development of the comprehensive omic ResolveDNA WGA and ResolveOME unified transcriptomic and genomic analysis platforms extend this capability. Key to realizing the impact of these chemistry systems is the ability to synergize with platforms developed to isolate and enrich for target cells that are key to deciphering the biological question.

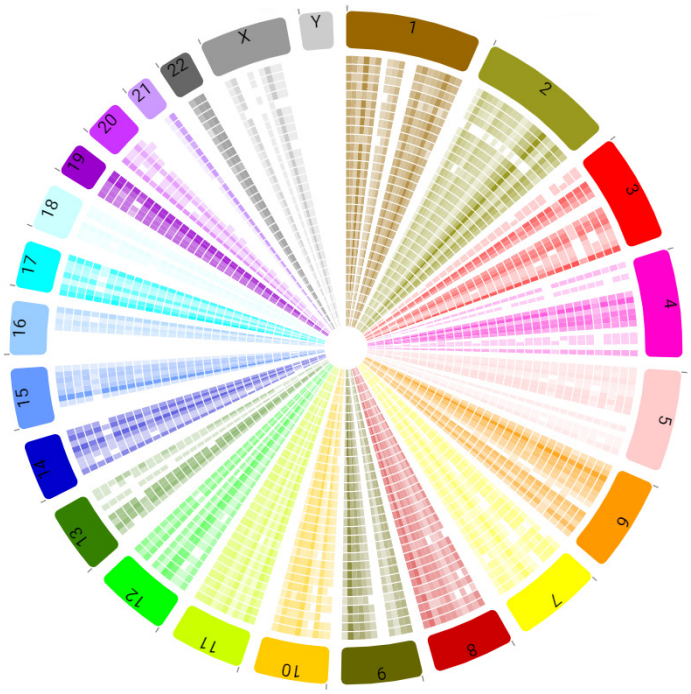


Figure 4. Variant density in BaseJumper: Variant density in BaseJumper: Quickly visually discern genome-wide differences in variant density between single cells (concentric rings) or zoom in for high-resolution regional ascertainment of variant density.

As described, there are a plethora of cell isolation and enrichment technologies available to the single-cell user today. To enable the greatest flexibility for the downstream user we have developed several example workflows to address a myriad of user needs. A central theme exists however, heterogeneity of tissue cells dictate the fate of the organism. This is true in both normal development, in the progression of disease, and the discovery of new and novel therapeutics. The integration of the BioSkryb Genomics comprehensive molecular omic platforms with these cell isolation and enrichment systems allows a new direction for the single cell researcher. Discovery is the ability to see something new for the first time. To facilitate this discovery, extending our methodologies beyond the existing systems is required.

Currently, genome-based analysis of single cells is limited by NGS sequencing throughput and cost. This is particularly true when sequencing whole genomes of single cells for SNV analysis. As an example, using the Illumina NovaSeq 6000, currently it is possible to sequence 30-40 single cells for comprehensive SNV calling. However, utilizing genome enrichment methods, either Whole Exome Sequencing (WES) or large targeted panels (~ 6700 genes) increases cellular throughput to several hundred to thousands of cells/flowcell sequencing run[8]. This flexibility in genome fraction analysis is critical as discovery depends on broad genome coverage of a wide array of implicated genes in various developmental pathways. Similar to increase in throughput of RNAseq methods, which was enabled by decreasing sequencing cost[9], we anticipate an ever growing need to increase cellular throughput for DNA analysis. As sequencing cost decreases, a proportional increase in comprehensive omic analysis will increase. The data will be evermore complex.

To interpret and understand such multi-faceted data sets, new data analysis tools will be required. For the ResolveDNA, ResolveOME and ResolveDNA Microbiome products this requires integration to the cloud-based analytics system BaseJumper (Figure 4). BaseJumper standardizes user views to focus on the discovery, while enabling flexible and user customized data analysis. The ability to interpret these complex data sets is, in the end, the most critical step in driving to impactful insights that can alter the course of disease in a broad array of application sectors including, oncology, neurology, reproductive health, immunology and influence of the microbiome on our environment and our health.

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*, ** Indicate methods in development at BioSkryb Genomics

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