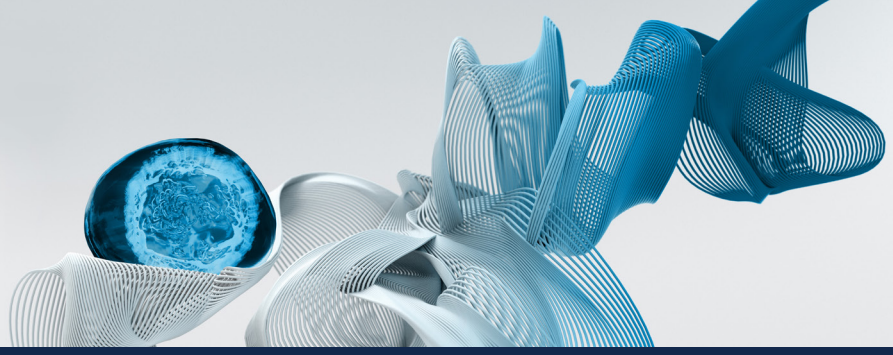
BioSkryb GENOMICS

Technical Note



Integrated Workflow for Spatial Single Cell Genome Analysis

Cells explored. Answers revealed.

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Integration of Spatial Cell Selection (CellCelector) with Primary Template-directed Amplification (ResolveDNA WGA) to Enable Spatial Single Cell Genomics

BioSkryb Genomics and ALS Jena have established a workflow to simplify the isolation of single cells from a variety of samples to enable low input and single-cell genomics. The workflow presented highlights how next generation single cell selection and sequencing technologies are able to obtain high quality single-cell genomic data from individual cells at a spatially specific location.

Introduction

Cellular heterogeneity dictates the fate of all tissues in both normal development and the pathogenesis of human disease. Defining this heterogeneity has primarily been focused on gene expression profiles in single cells[1,2]. While expression based analysis is highly valuable for defining variable cell populations, actionable information on therapeutic selection for oncology and potentially other fields like neurology depends on the highest resolution genome data possible.

The limitation of attaining high definition genome data from single cells has been gated by both the ability to isolate the critical cells of interest and the ability to amplify the genome with high uniformity and complete coverage. By combining the ability to select individual cells from a surface, and transfer these cells to a reaction vessel, such as a microtiter plate, we have developed a workflow to spatially locate a cell of interest, capture that individual cell[3] and amplify the genome to enable Next Generation sequencing (NGS) analysis of the genome at the highest possible data quality[4]. In summary, the combined platform allows for spatial selection and capture of individual cells of interest using the CellCelector platform and deeper interogation of the genome of each cell using the ResolveDNA NGS workflow (Figure 1). Together this combined platform allows the user to define genomic heterogeneity in any sample type.

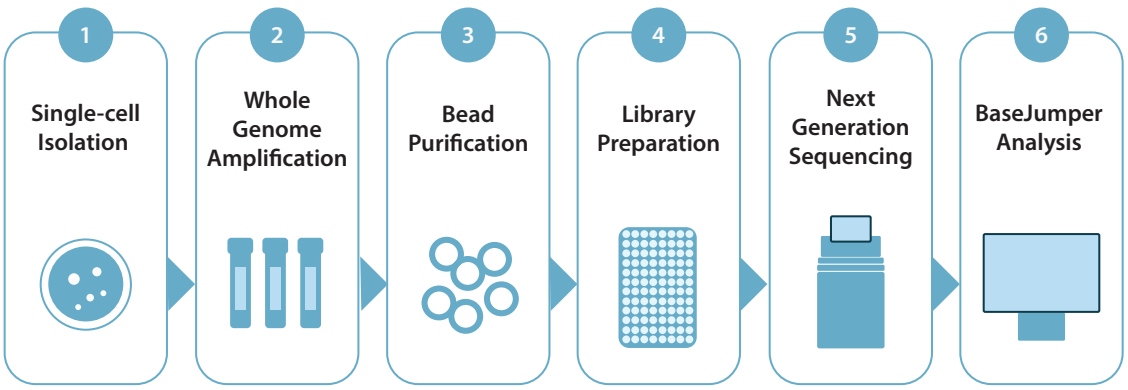

Figure 1. CellCelector™ and ResolveDNA™ Workflow: CellCelector isolated single cells undergo Primary Template-directed Amplification, followed by library preparation, sequencing, and analysis with BaseJumper software.

The integrated platform overcomes challenges of single cell genome sequencing

- Gently & accurately isolates intact single cells (Figure 2 & 3)
- Obtaining sufficient genomic material for downstream analyses
- Low and variable genomic coverage
- Amplification artifacts such as allelic bias, mutations, and chimeras.

Integrated CellCelector ResolveDNA NGS Platform Benefits

Upstream- ALS Jena Single-cell CellCelector:

- Image (brightfield/fluorescence) based single-cell selection
- Selection of suspension and adherent cells with image-based capture assessment and storage
- Single cells from samples containing < 10K cells (i.e. fine needle aspirate)
- Cell selection from microwell plates, slides, nanowell arrays, and polymer gels (colony picking)
- Gently and accurately isolates intact single cells
- Confidently avoids contamination concerns
- Easily integrates into any biologic experimental workflow

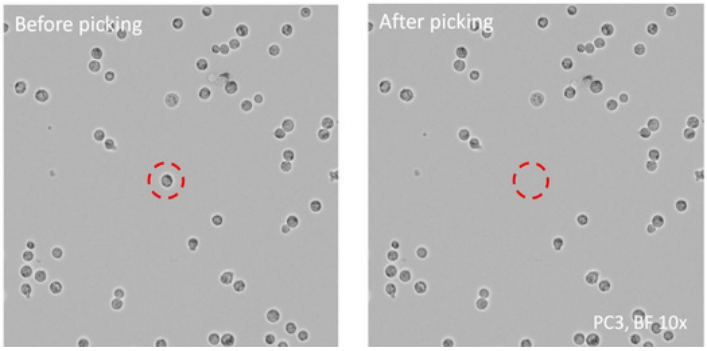

Figure 2. Verify single-cell capture. The automated workflow provides a live image during picking including cell tracking, plate to destination plate data as well as images before and after picking.

Downstream- BioSkryb's ResolveDNA™ NGS Platform:

- Directly copies single-cell genomes or low-input DNA with Primary Template-direct Amplification[5]
- Amplifies with unprecedented genomic coverage uniformity and breadth (Figure 4)
- Precisely thwarts error propagation with high allelic balance
- Collectively, enables hitherto unachieved confidence in single nucleotide variant (SNV) and copy number variation (CNV) calling from single cell

Spatially Isolate Intact Single Cells with the CellCelector

EASILY image the entire sample to determine cells of interest for selection. Selected suspension or adherent single cells are then captured and automatically placed in a discrete volume within a collection tube or in a 24, 96, 384 or 1536 well plate.

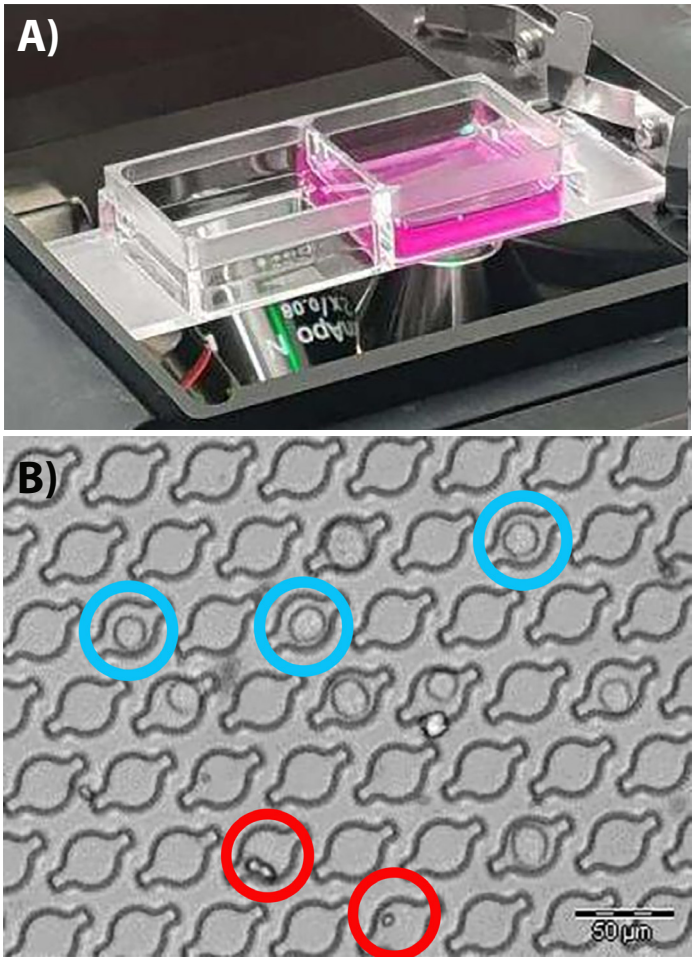

Figure 3. Highly accurate isolation of dissociated cells. Using the CellCelector, isolation of rare cells or cells at low density concentration can be accomplished using a nanowell array. These arrays, containing up to 300K capture wells, provide the ability to first qualify a cell (blue circles) or reject a debris object (red circles) prior to isolation and capture. The process is very useful to isolate cells from precious clinical samples where often the total cell number is below 10,000 cells.

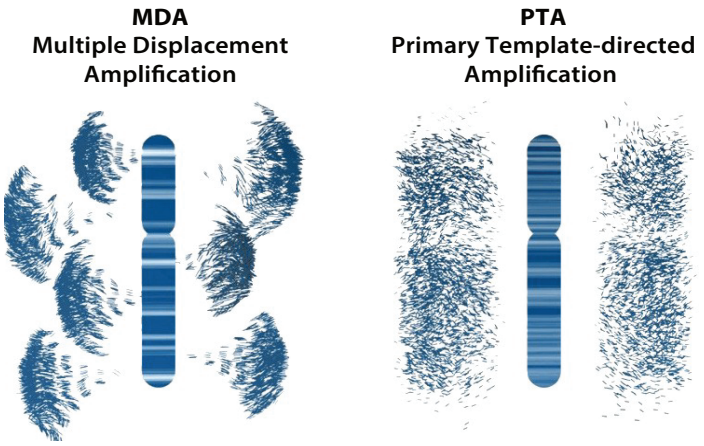

Figure 4. Schematic of genomic amplification output obtained from MDA vs PTA. PTA prevents exponential read pileup and error propagation and yields highly uniform coverage.

CONFIDENTLY avoid contamination from previous experiments with disposable cell isolation capillary tips and fluidics. Various routines can be programmed to ensure the capillary tip is free of carryover material from sample to sample or single cell-isolation to single cell-isolation. Use pre-loaded well-specific reagents, or alternatively use a common reagent well for all cell selection to increase cell capture speed.

GENTLY isolate cells-of-interest without shear force and with low pressure. Avoid worrying about changes to cell viability and sequence quality.

RELIABLY capture all cells by image based verification (Figure 2). Confirm each cell has been removed from capture surface and that the isolated cell has been deposited in the correct location by imaging the capture vessel (when using the flat bottom collection tubes).

FLEXIBLY capture from a variety of sample collection devices including microwell plates (Figure 3A), solid media, nanowell arrays and culture plates (Figure 3B).

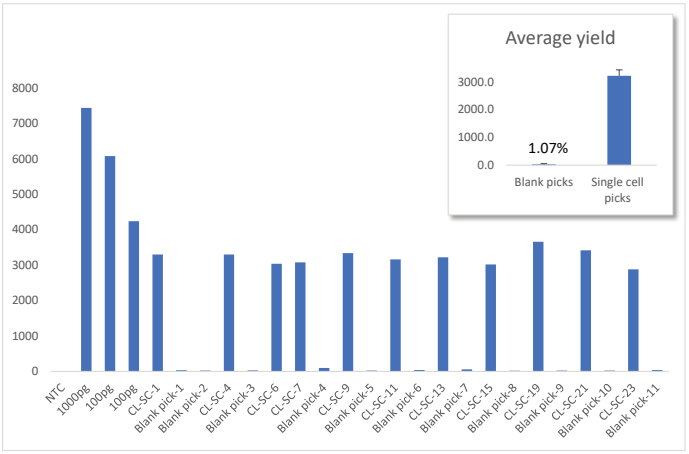

Figure 5. Amplification efficiency of CellCelector isolated single cells. Viable GM12878 cells were isolated into 96-well plates with BioSkryb Cell Buffer and amplified by ResolveDNA. We detect low cross contamination (~ 1.07%) between selection of individual cells as assessed by selecting regions absent of cells.

Study Single-cell Genomes with ResolveDNA NGS Workflow

DIRECTLY copy the primary template of selected cells with an isothermal polymerase and proprietary termination chemistry that attenuates the size of amplicons. The smaller amplicons do not efficiently amplify, so randomer primers are redirected to the primary template of interest (Figure 4).

PRECISELY amplify low-input DNA and single-cell inputs to reproducibly capture >95% of the genome without cross contamination between cell isolations (Figure 5).

UNIFORMLY amplify with high breadth of coverage, few replication errors, and low allelic bias[4], to call single nucleotide variants (SNV) at the whole genome sequencing (WGS), whole exome sequencing (WES), and small-panel levels. More information is available on these workflows at bioskryb.com.

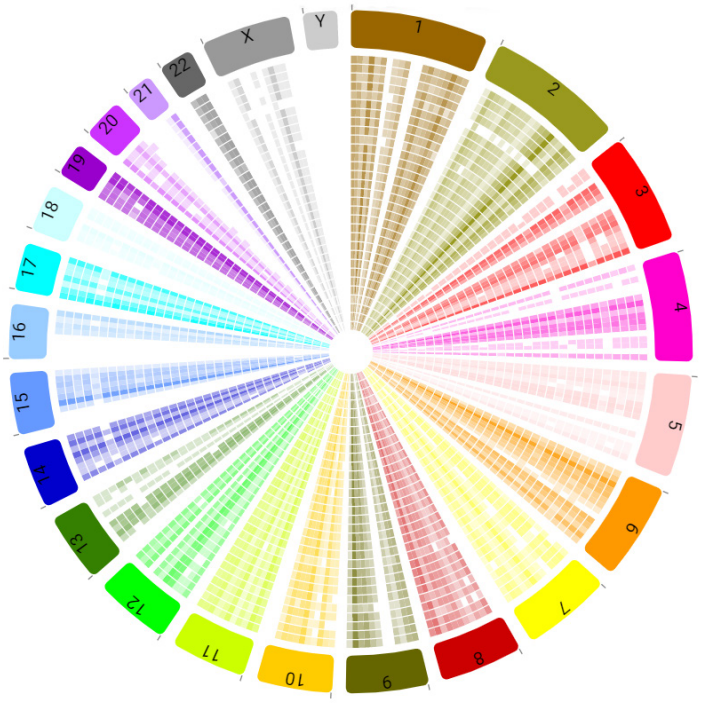

Figure 6. Circos plot browser in BaseJumper: Explore variant density and ancestral variation at different levels of resolution.

Construct a Library Directly with PTA Amplified Genomes

ROBUSTLY construct a library using PTA product as input with a choice of library construction protocols. Only 100 ng of PTA product is required as input with the ResolveDNA™ Library Preparation Kit from BioSkryb. The workflow uses a ligation-based workflow that does not require fragmentation of the input DNA and utilizes unique dual-index adapters that are compatible with Illumina sequencers.

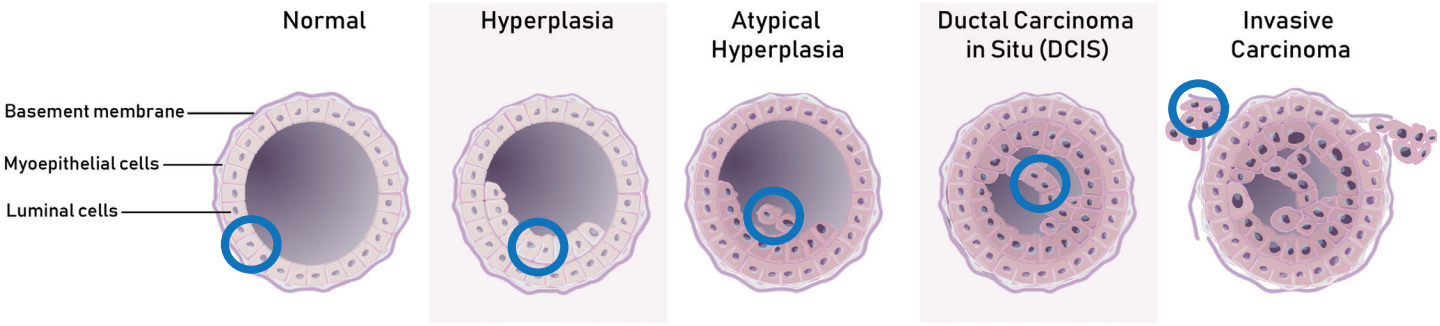

Figure 7. Spatial isolation of specific cells within the heterogeneous temporal tumor environment. Histopathology is a critical tool for understanding the evolution of disease. Using the example of breast ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) transition, normal cells begin to modify their characteristics which lead to invasive and metastatic cancer. By developing the ability to isolate these individual cells (blue circles), and interrogate the genetic abnormalities that arise, a clear understanding of the influence of the genomic modifications can be assessed. Capturing this spatial and temporal data in the context of tumor evolution, which is possible within a single tissue sample, allows the assessment of new strategies to overcome therapeutic failure.

ResolveDNA WGA products can also be used in the KAPA HyperPlus construction protocol, though at a higher 500 ng input. Alternatively, directly tagmented PTA products can be used with Illumina DNA Prep reagents. All workflows yield >400 ng of amplified library, facilitating downstream enrichments if necessary.

Sequence and Analyze with BaseJumper

QUICKLY perform low-depth sequencing on any Illumina instrument to obtain QC sequencing metrics prior to performing alignment (Table 1).

SIMPLY analyze high-depth sequencing, align and call variants. Visualize variant call file data with BioSkryb's BaseJumper Bioinformatics platform (Figure 6). Single nucleotide variation can be explored in the context of a lineage browser—to explore variants that may have occurred ancestrally.

Table 1. QC Analysis of ALS Jena CellCelector-BioSkryb ResolveDNA Workflow

QC Metric	Value
PreSeq Count	$3.50 (\pm 0.02) \times 10^9$
Alignment	0.978 ± 0.001
Chimeras	0.106 ± 0.007
ChrM	0.01 ± 0.005

Acquire the combined ALS Jena CellCelector and BioSkryb Genomics NGS workflow using our Early Access Program

Heterogeneity within cellular populations drives the underlying biology of life. Using the unprecedented ability to select and isolate cells from a specific positional location we are developing the capability to associate the spatial and temporal nature of the genomic signature that influences tissue organization in normal development and pathology. We welcome the opportunity to work collaboratively with our user base to develop a new suite of genome based spatial analysis tools through our CellCelector early access program.

Summary

At a fundamental level for oncology, the ability to select cells from a specific location or region within a tissue, and analyze each discrete genome allows for the understanding of tumor heterogeneity in a spatial and temporal context. This evolution from the founding clone, through a pre-cancerous lesion, to an oncology based pathology (figure 7), is rooted not only in the menagerie of various cells, but in the way they interact with each other, the tissue ultrastructure as well as the circulatory and lymphatic systems. At BioSkryb Genomics we are exploring these questions and developing new applications to answer these foundational questions in the fields of oncology, reproductive medicine, neurology and immunology.

We welcome the interaction of our user base to fuel new discoveries that impact patient care. We welcome you to grow with us and evolve your research program.

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Published by:

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TAS.029 03/2022