Whole Genome and Full-Length Transcriptome from the Same Single Cells using ResolveOME

Whole Genome
Full Length Transcriptome
Same Single cell

xinkun.wang@northwestern.edu
Director, NUSeq Core Facility

John Bon – john.bon@BioSkryb.com
Christina Ulrich – Christina.Ulrich@BioSkryb.com
Andrew Rohs – Andrew.Rohs@BioSkryb.com

Agenda

- BioSkryb Genomics- Single-Cell Multiomics
- **ResolveDNA®**
  - WGA with great genomic coverage and uniformity for **SNV** and **CNV analysis** from the same single cell
- **ResolveOME™**
  - a unified system for **single-cell full-length mRNA transcriptome**, **whole genome amplification** and **NGS library preparation** for sequencing
- Kits to do wet lab in your own laboratory, core lab or services lab at BioSkryb
- **BaseJumper™** - Data analysis solution
- Workflows and data

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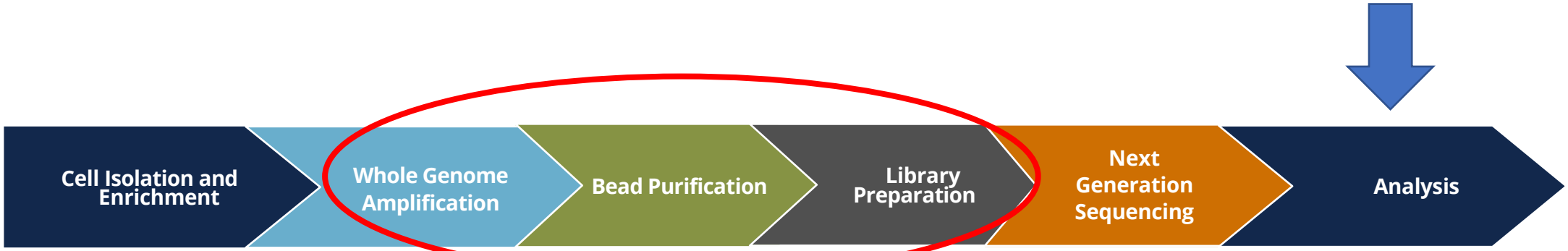
End-to-End Single-Cell Omics Workflow



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Complete Starter Pack

ResolveOME™
&
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Whole Genome Amplification Kit

ResolveOME™
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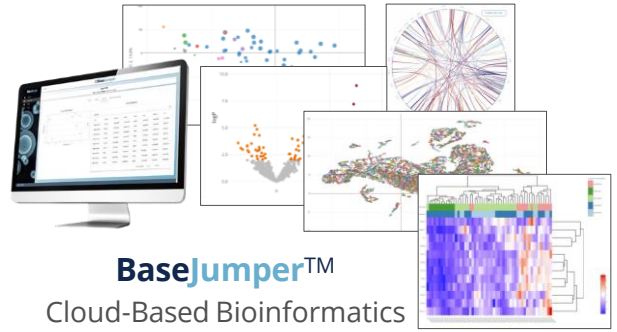

BaseJumper™
Cloud-Based Bioinformatics

Whole Genome or Whole Exome SEQ

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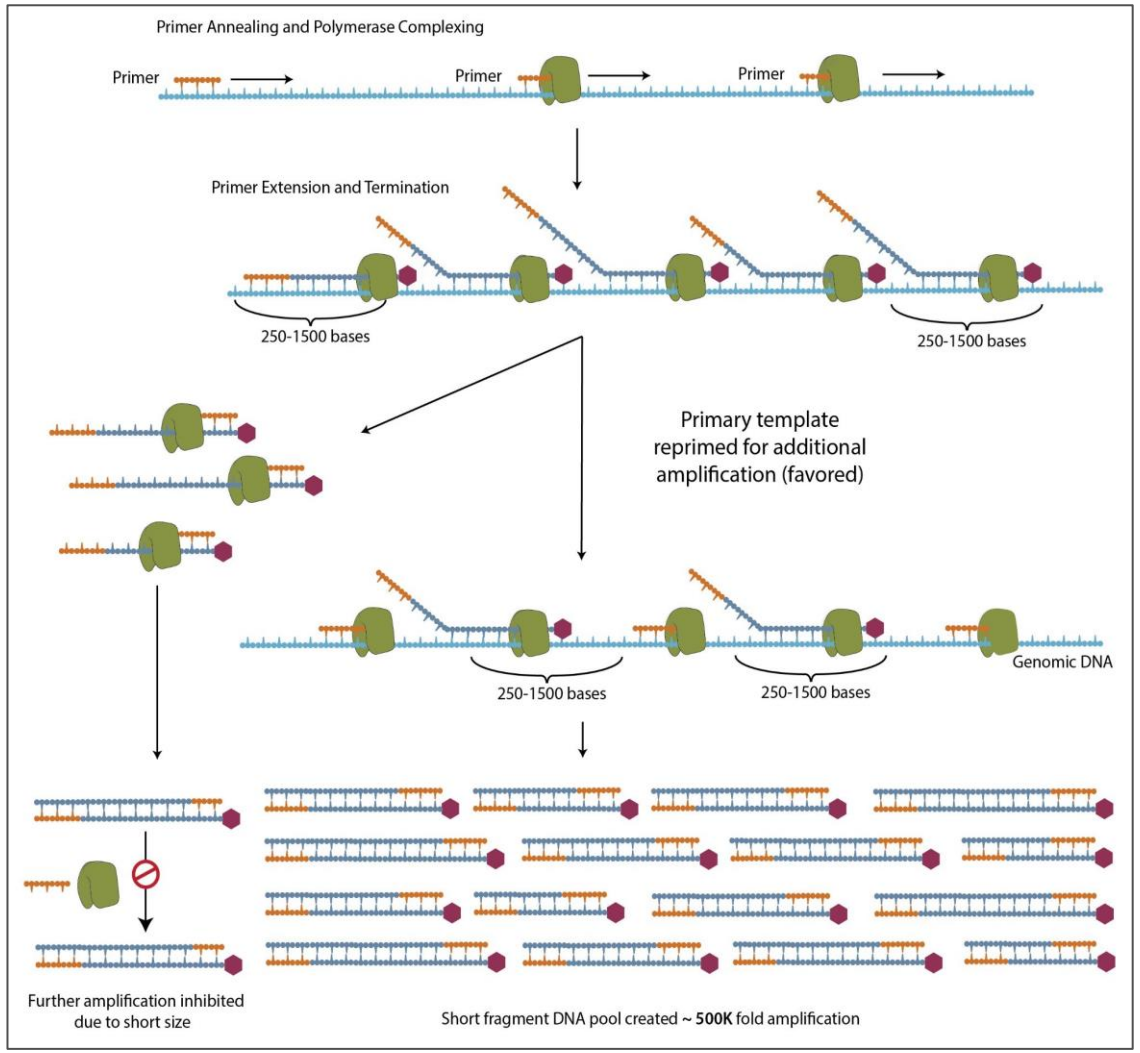
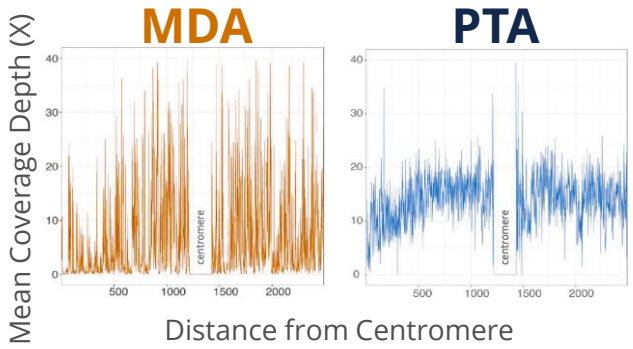
The Technology Underpinning BioSkryb:
Primary Template-Directed Amplification (PTA)

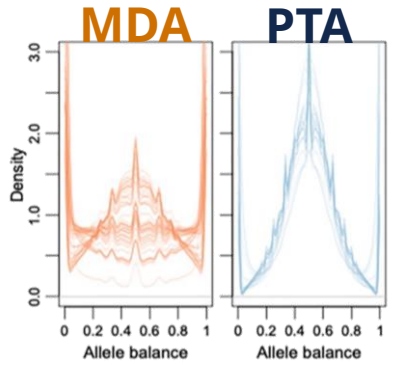


More Uniform Genome Coverage



Gonzalez-Pena et al. 2021 PNAS

Superior Allelic Balance



Luquette et al. 2021 bioRxiv

- **Enables high resolution variant calling**
  - **CNVs, SNVs, structural variants**
- **improved uniformity & coverage, reproducibility and variant call rates**

ResolveDNA - ResolveOME enables comprehensive, unbiased, and precise whole genome sequencing from a single cell.

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ResolveDNA Performance Characteristics

Method	ResolveDNA	Mixed Method A	MDA A	MDA B	Mixed Method B	Mixed Method C	DOP-PCR
Genome Mapping	97%	91%	88%	55%	88%	55%	52%
Genome Recovery	97%	73%	65%	59%	50%	33%	20%
CV of coverage	0.8	1.3	1.8	2.3	2.6	3.2	3.6
SNV sensitivity	92%	70%	65%	55%	45%	30%	19%
SNV Precision	99%	88%	87%	88%	28%	35%	35%

Sensitivity are based on positions that are 25X coverage for ResolveDNA, Values for alternaive methods taken from Gonzalez, etal. PNAS[1]
CV: Coefficient of Variation SNV: Single Nucelotide Variant

ResolveDNA outperforms other common methods with respect to data quality and variant calling metrics

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Case Study: Age-related Neuronal Mutation Accumulation

Indels in gene-regulatory elements have a considerable effect on genome integrity in human neurons

HARVARD MEDICAL SCHOOL

nature genetics

TECHNICAL REPORT

https://doi.org/10.1038/s41588-022-01180-2

Single-cell genome sequencing of human neurons identifies somatic point mutation and indel enrichment in regulatory elements

Lovelace J. Luquette[1,16], Michael B. Miller[2,3,4,5,6,16], Zinan Zhou[2,16], Craig L. Bohrson[1], Yifan Zhao[1], Hu Jin[1], Doga Gulhan[1], Javier Ganz[2], Sara Bizzotto[2], Samantha Kirkham[2], Tino Hochepied[7,8], Claude Libert[7,8], Alon Galor[1], Junho Kim[2,9], Michael A. Lodato[10], Juan I. Garaycoechea[11], Charles Gawad[12,13], Jay West[14], Christopher A. Walsh[2,3,15,17] ✉ and Peter J. Park[1,17] ✉

- Indels accumulate slowly, requiring single cell techniques sensitive to characterize **low individual cell mutational burden**
- **ResolveDNA** is used because it **provides sensitivity** and eliminates false discovery rates for single-cell SNV, CNV and Indels analysis, **ideal for characterizing single-cell mutational burden**
- ResolveDNA is ideal for **elucidating mechanisms of action for progressive pathologies such as Alzheimer's Disease** and other neurological disease investigation

Luquette et al., Nature Genetics 2022

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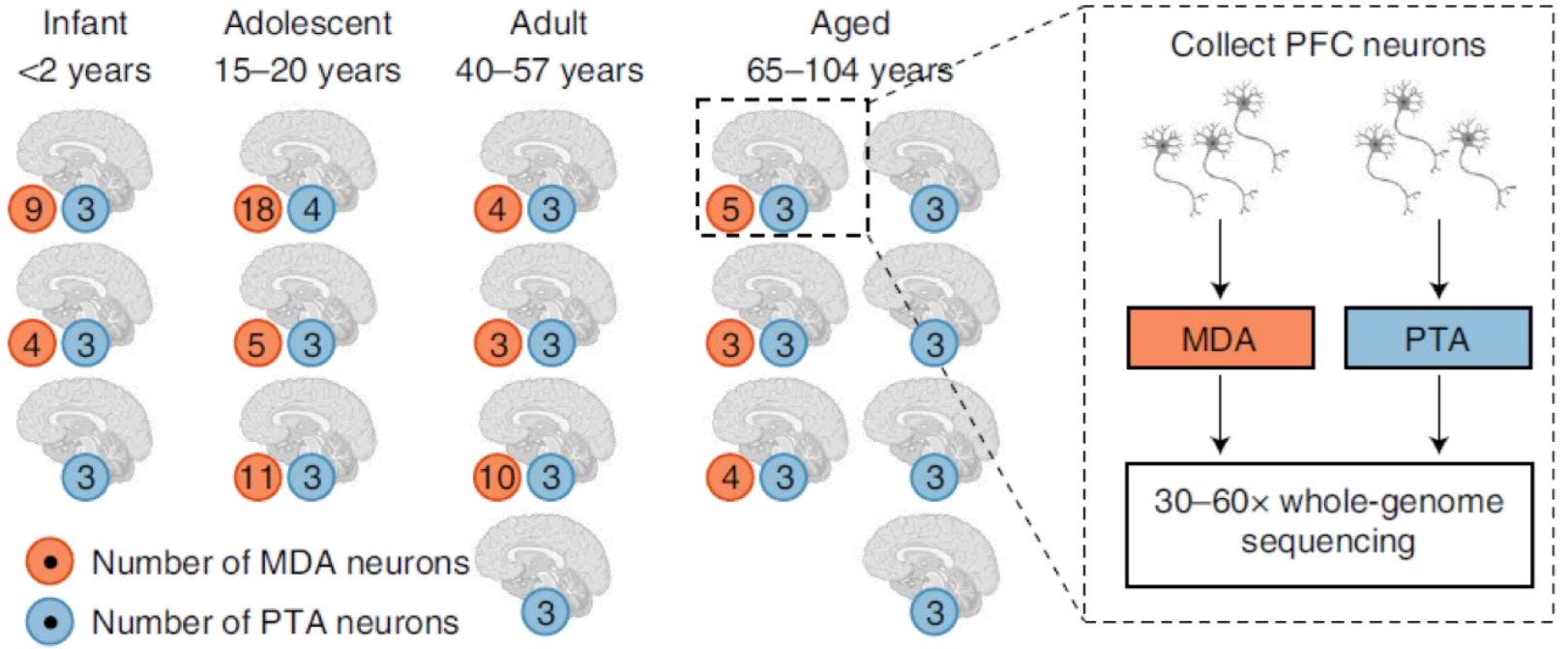
Processing Pre-frontal Cortices (PFCs) with ResolveDNA

- Nuclei from single neurons were collected from the PFCs of brains of 17 individuals ranging in age from infancy to elderly
- Single nuclei was amplified by using ResolveDNA (PTA) or multiple displacement amplification (MDA) chemistry and sequenced to high coverage
- To determine chemistry utility large scale characteristics such as copy number variation, amplification uniformity and allelic balance was assessed



Luquette et al., Nature Genetics 2022

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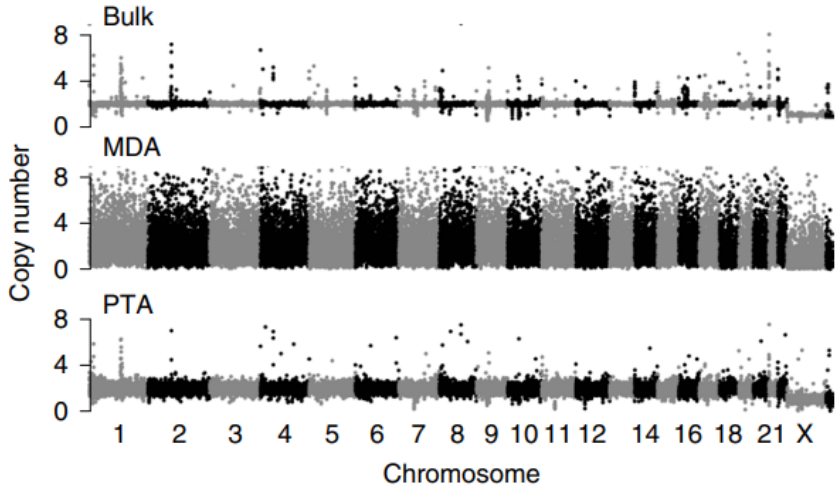
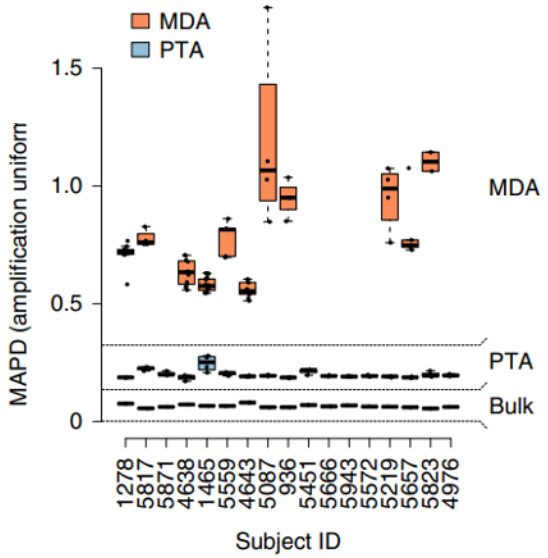
Characteristics of ResolveDNA in Single Nuclei from PFCs

Copy number variation



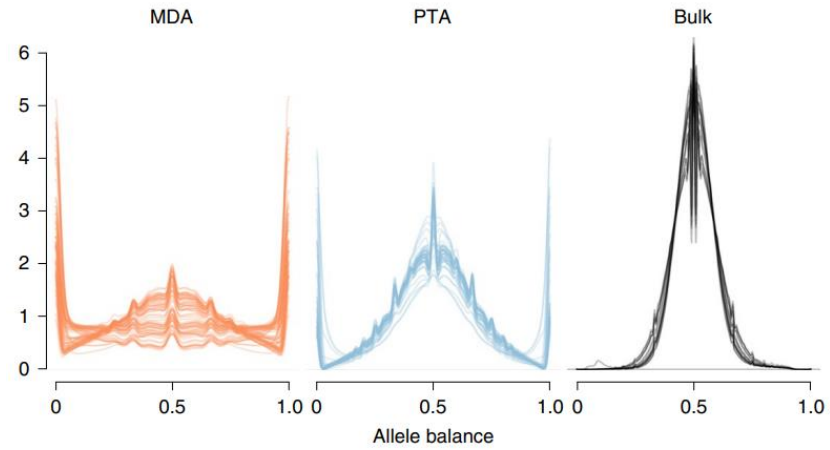
ResolveDNA yields superior accuracy in copy number analysis compared to MDA.

Amplification uniformity



ResolveDNA and MDA-amplified neuronal genomes show **significantly improved amplification uniformity** via ResolveDNA and PTC compared to MDA.

Allelic balance



For germline heterozygous SNPs the evenness of amplification was measured between homologous alleles in a diploid cell. On average, 71% of each ResolveDNA PTA genome was balanced compared with **only 39% of each MDA genome.**

Luquette et al., Nature Genetics 2022

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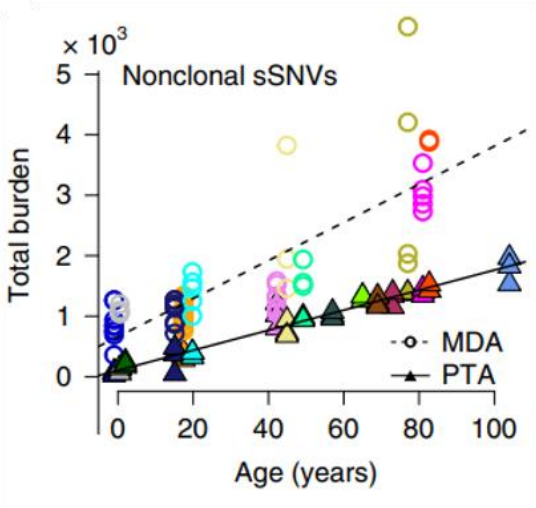
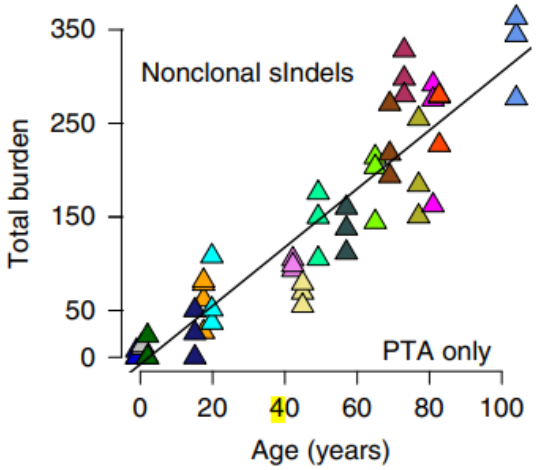
PTA Outclasses MDA-based Approaches in somatic SNV & InDel



Genome-wide, extrapolated accumulation rate of **somatic SNVs** in PTA- (triangles) and MDA- (circles) amplified single human neurons.

Somatic SNV sensitivity
Total rate of SNVs in single human neurons exposes sensitivity issues in MDA coverage, caused by FDR from amplification artefacts.



Genome-wide extrapolated rate of **somatic indel** accumulation.

Somatic Indel sensitivity
"As was the case for SNVs, MDA yielded a higher accumulation rate estimate of 6.0 somatic indels per year and we again attribute this to MDA artifacts"

Luquette et al., Nature Genetics 2022

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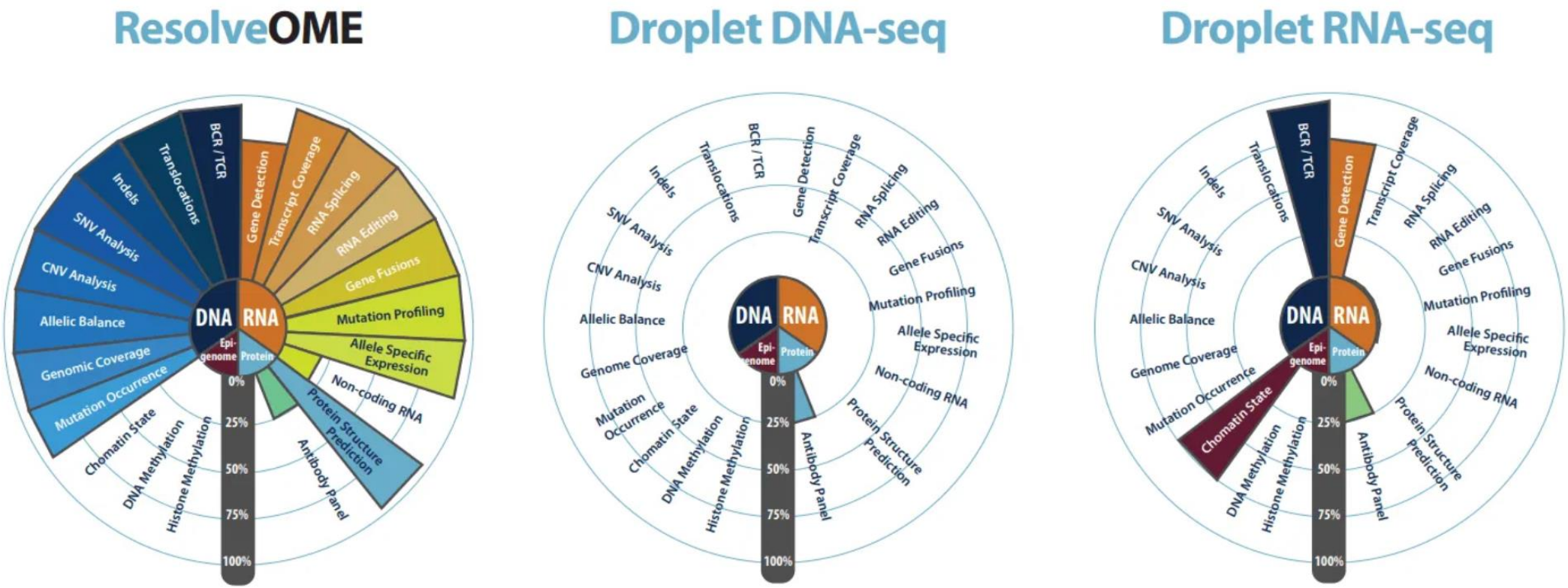
ResolveOME

Enabling Comprehensive Single-Cell Multiomic Analysis



ResolveOME provides >3 fold more data per single-cell than droplet methodologies and provides a comprehensive view of single-cells important to drive actionable multiomic analysis.

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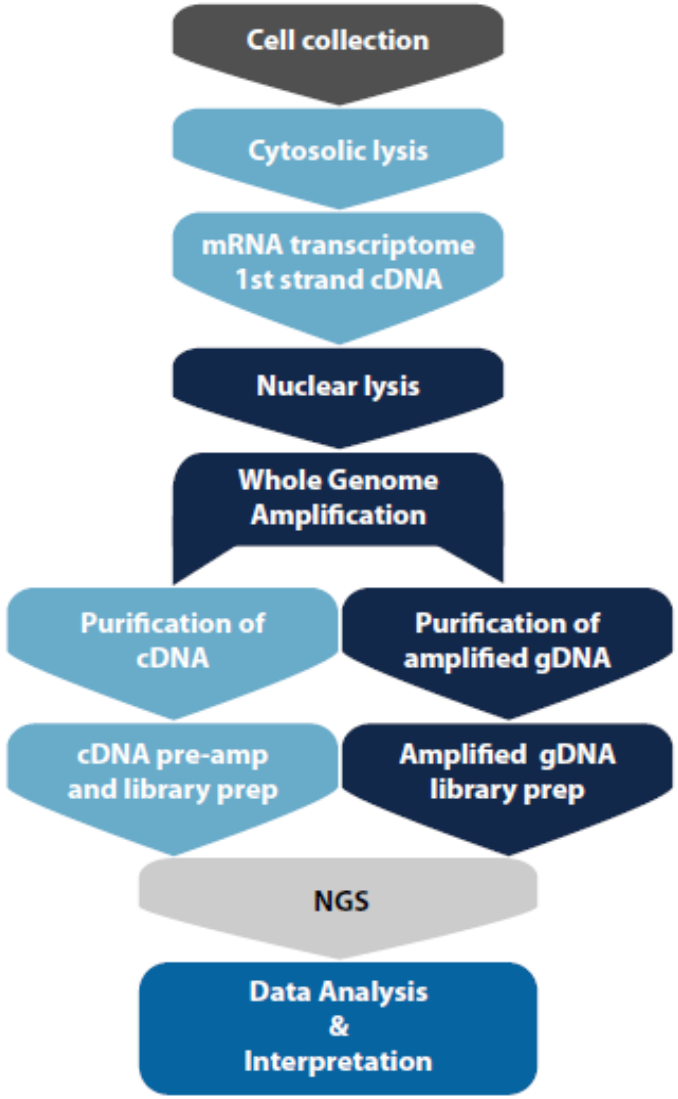
ResolveOME Workflow Overview

Unified workflow enables DNA and RNA analysis from the same cell

- Cells can be collected by any means (e.g., FACS, LCM, cell culture, frozen 96 or 384 well plates).
- Optimum input is 4pg or single cell and is applicable to both single-cell and bulk sample inputs.
- Entire ResolveOME workflow is completed in 2-3 days from cell sorting to sequencing-ready libraries.
- Generally, the workflow is adopted complementary to droplet-based methodologies looking at a few thousand cells (vs hundreds of thousands of cells) as ResolveOME is offers more in-depth data per single-cell



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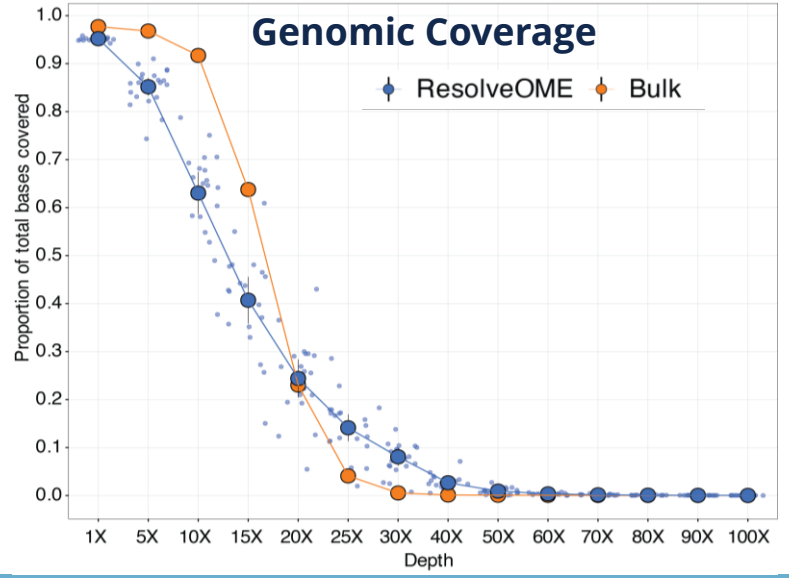
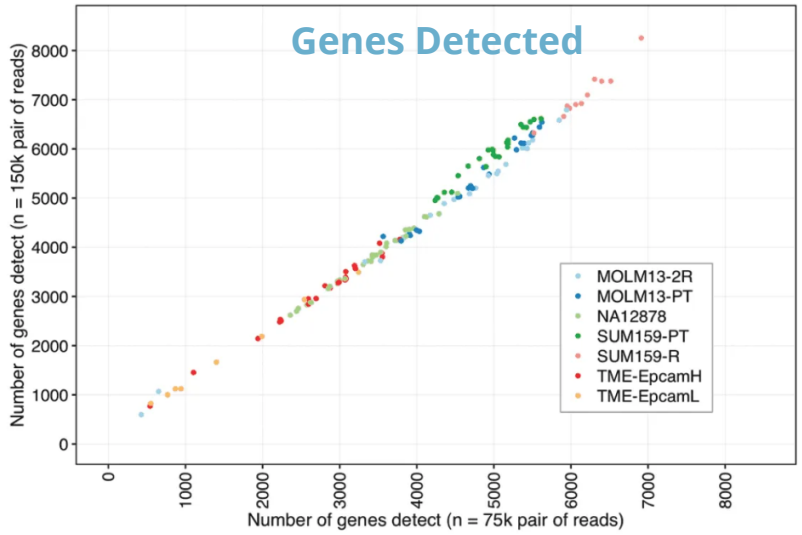
ResolveOME: A Single Cell's *Most Complete* Genome and Transcriptome

DNA Performance Characteristics	Observed Values
Accuracy	99.5%
Sensitivity	97.1%
Specificity	99.2%
Precision	99.5%
Allelic Balance	98.4%
Genome Coverage	97.1%



RNA Performance Characteristics	Observed Values
Genes Detected	4,546 Genes*
Reportable Range	6057
Average Concordance	0.91
Reproducibility (CV)	43.3%



**NA12878/HG001 cells*

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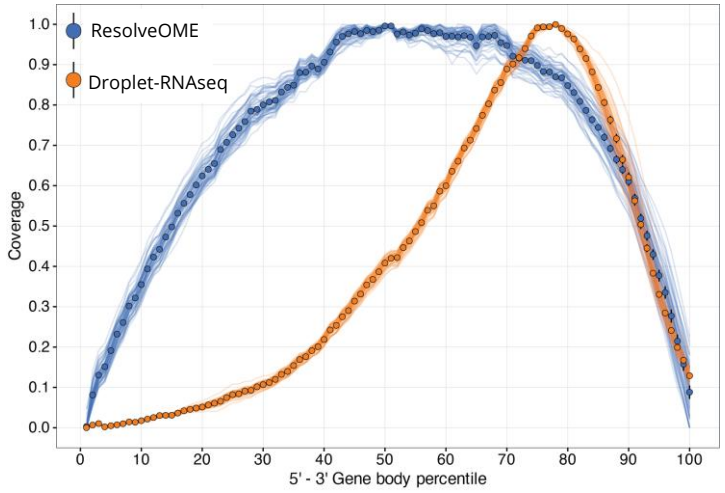
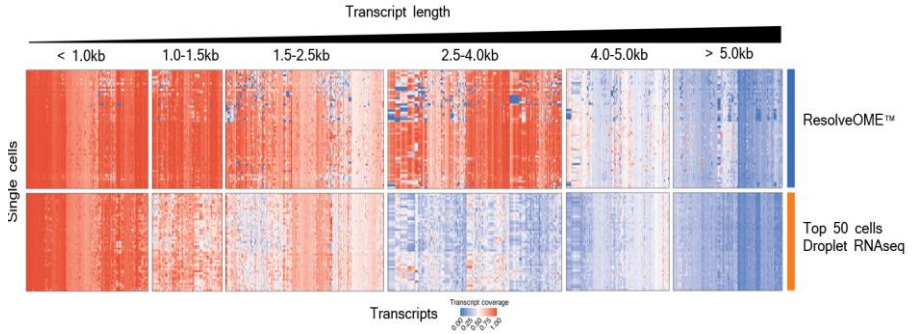
ResolveOME Transcriptome Amplification

The industry-leading performance of ResolveOME coupled with superior transcriptome capture enables genomic and transcriptomic analysis from an individual cell.

Superior RNA Performance: Enhanced Gene Body Coverage



Superior RNA Performance: Increased Representation Across Transcript Sizes



**Comparison versus droplet-RNAseq. All data on file.*

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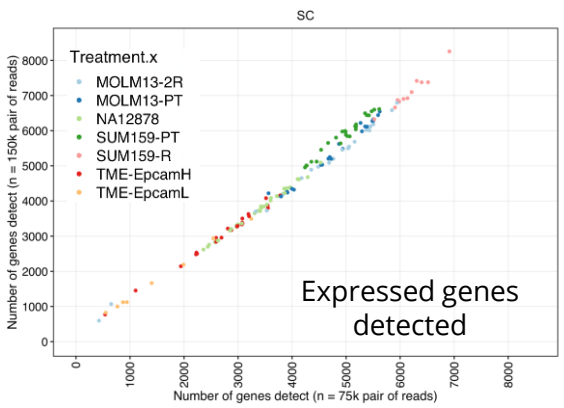
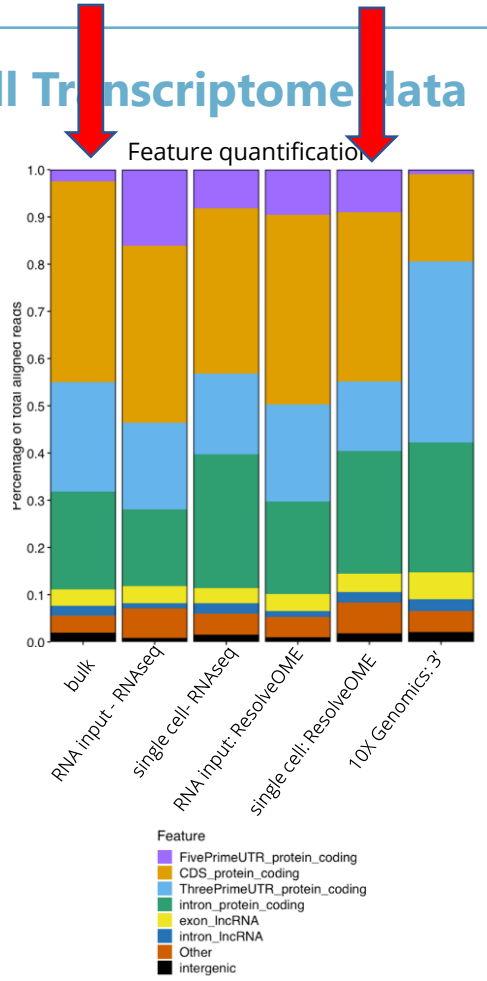
ResolveOME High Quality Transcript and Genome Data

ResolveOME Single-cell Transcriptome Data





- Absent Genome contamination
- Detection of up 7K genes
- High dynamic range
- TCR/BCR analysis
- Full length transcripts allowing
  - Splice Variant
  - Fusion gene
  - Truncations
- Extendable to targeted proteins

More information on our bioRxiv paper Zawistowski et al., 2022

- Full-length detection of transcriptomic features including 5’ and 3’ UTR overcoming the bias often observed with end counting methodologies
- Detection of ~7,000 genes per single-cell
- More in depth expression analysis and variant detection in the mRNA
- Splice variants, SNVs, fusion genes and other structural variants detected with full length transcriptome analysis

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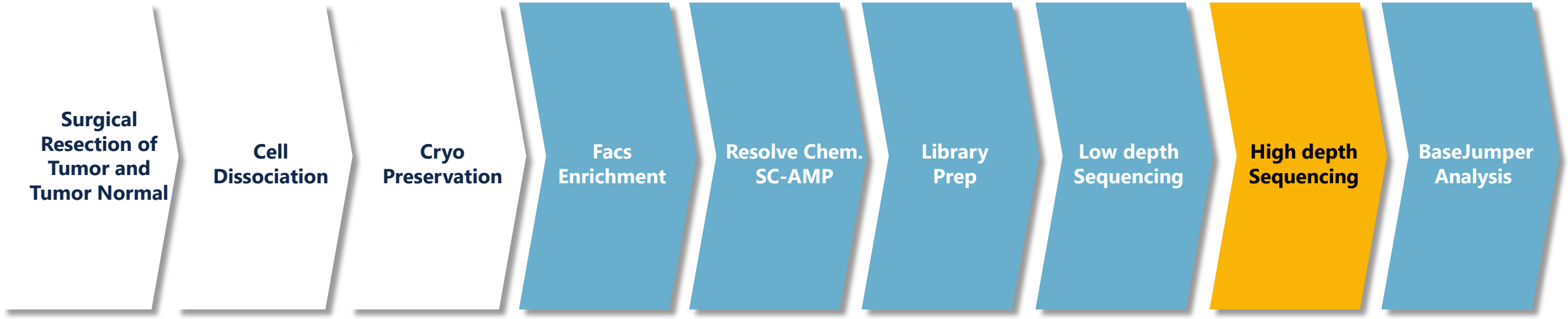
Ductal Carcinoma in Situ (DCIS) Collaboration – Customer study



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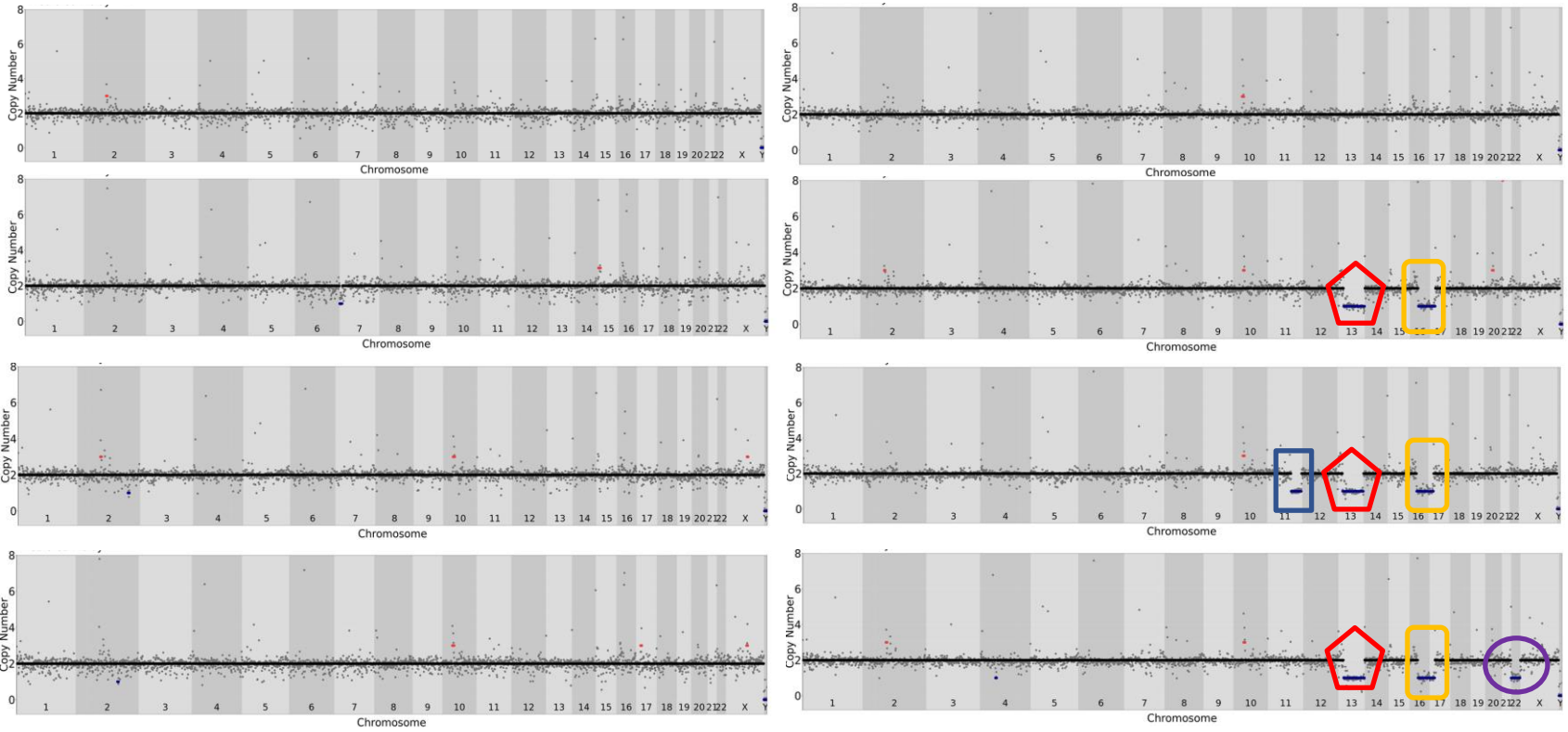
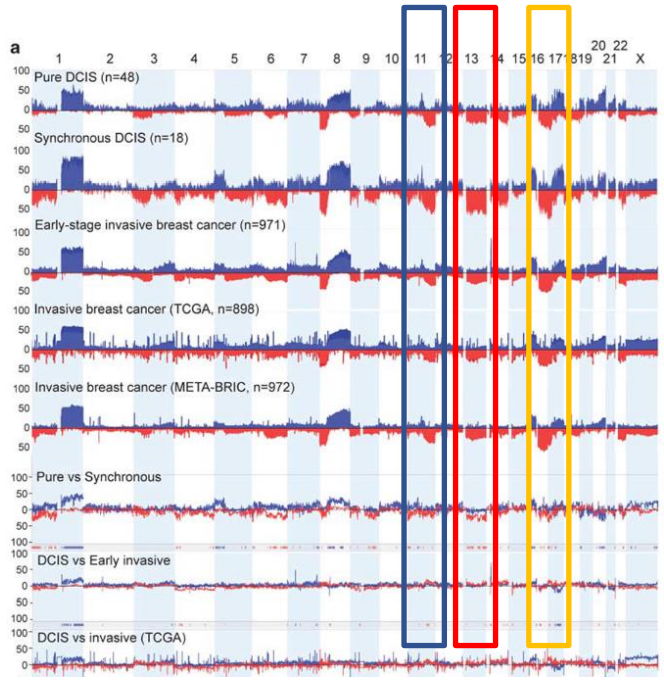
CNV analysis: Patient TME-19-016

Normal breast single cells

Tumor single cells





Copy number analysis of ductal carcinoma *in situ* with and without recurrence

Kylie L Gorringe, Sally M Hunter, Jia-Min Pang, Ken Opeskin, Prue Hill, Simone M Rowley, David Y H Choong, Ella R Thompson, Alexander Dobrovic, Stephen B Fox, G Bruce Mann & Ian G Campbell

Modern Pathology **28**, 1174–1184 (2015) | Cite this article

Ginkgo, 2.5 Mb windows, 5 million PE read subsampling

Chromosome: 11q deletion

Tumor suppressor: 13 deletion *BRCA2; RB1*

16q/17p deletion *TP53*

22 deletion

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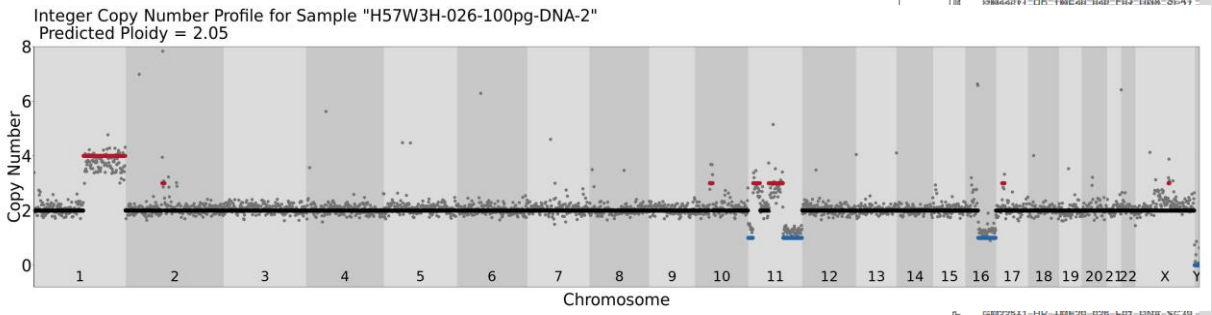
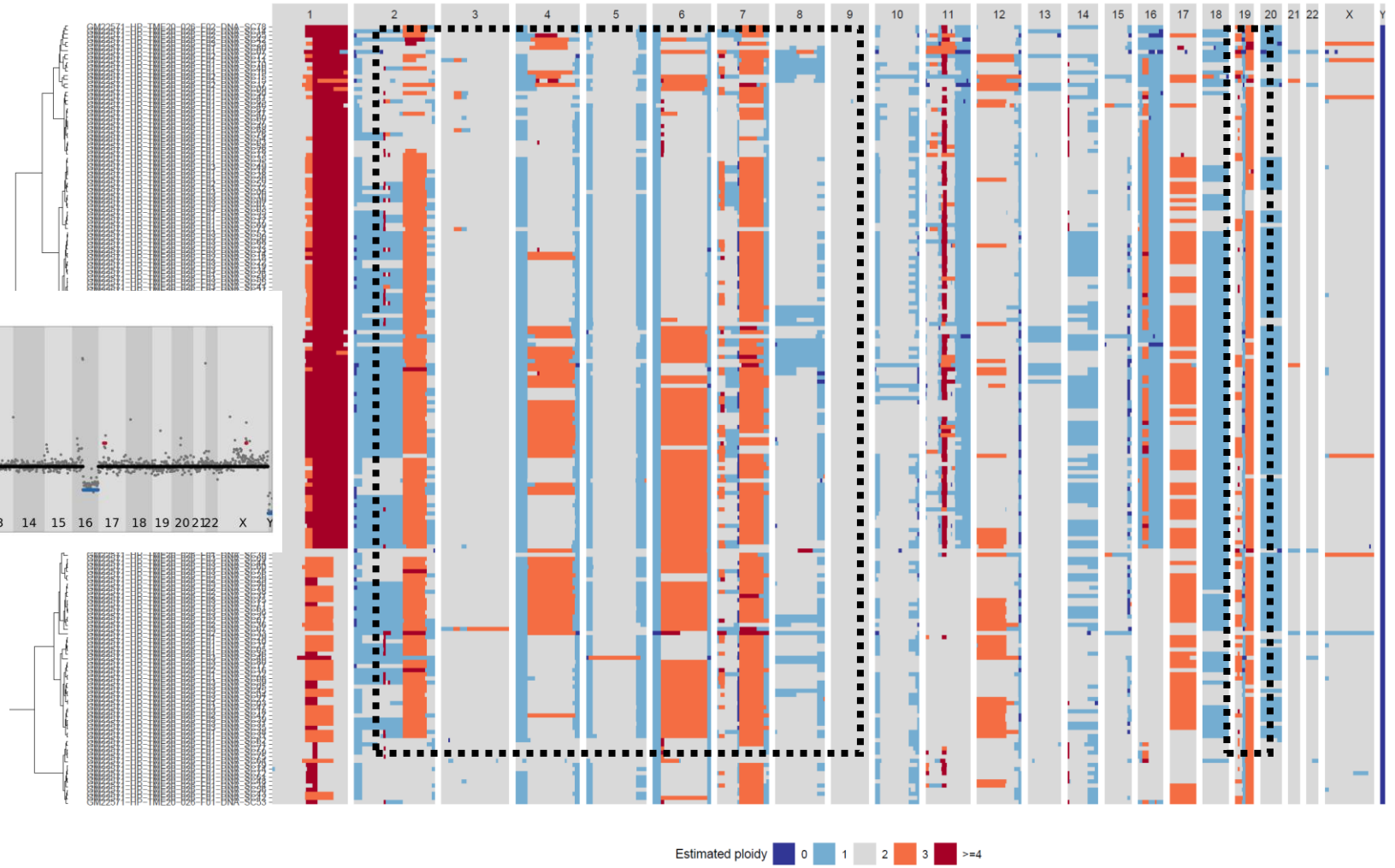
Copy Number Insights Are Missed by Bulk Sequencing

…but misses other discrete alterations in EpCAM enriched cells

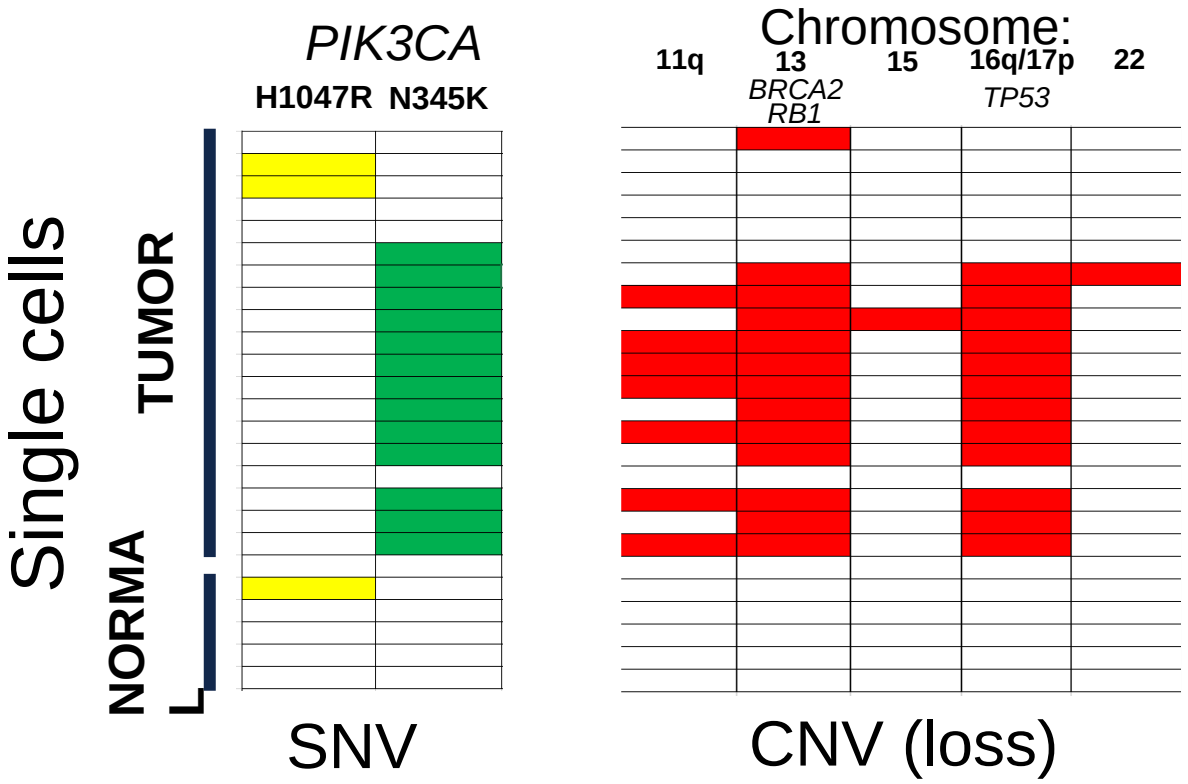
Bulk captures some predominant alterations seen in single cells…



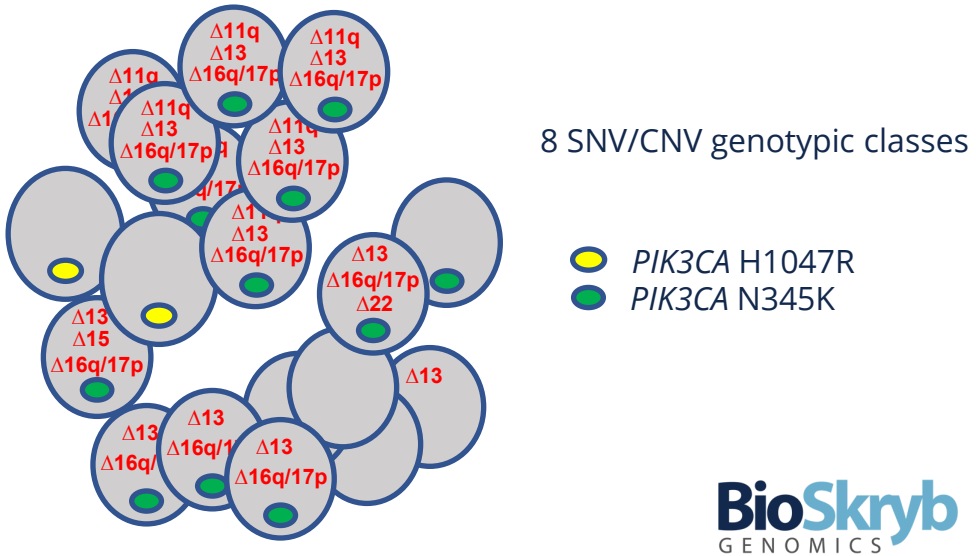
Diversity of cell types in a tumor sample mutes rarer alterations



Integration of *PIK3CA* mutation status and copy number loss in single cells



- Striking single cell heterogeneity revealed, even with 19 tumor cells from one patient
- SNV / CNV interplay! Known interaction between p53 and PI3 kinase; Rb and PI3 kinase. Some cells had a PIK3CA variant but no CNVs.
    - These cells may be pre-malignant



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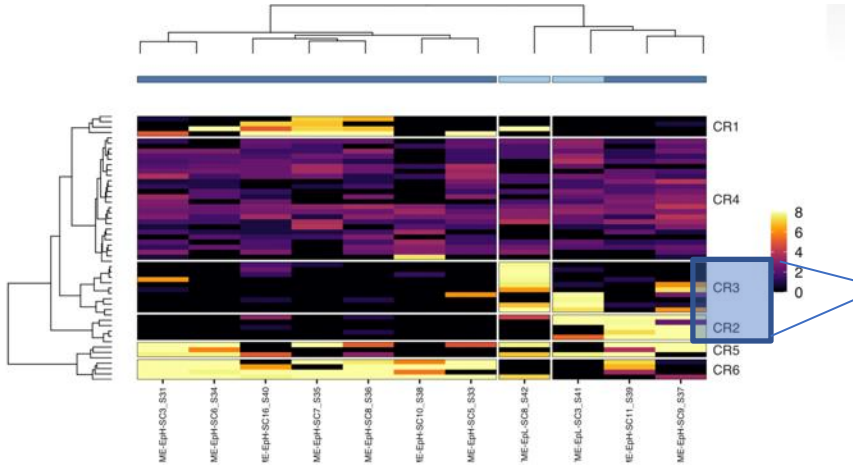
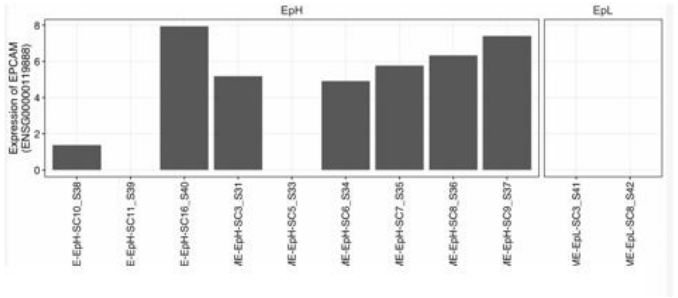
Immune signatures- EpCAM low cells: top 50 variable genes

Cell Identity

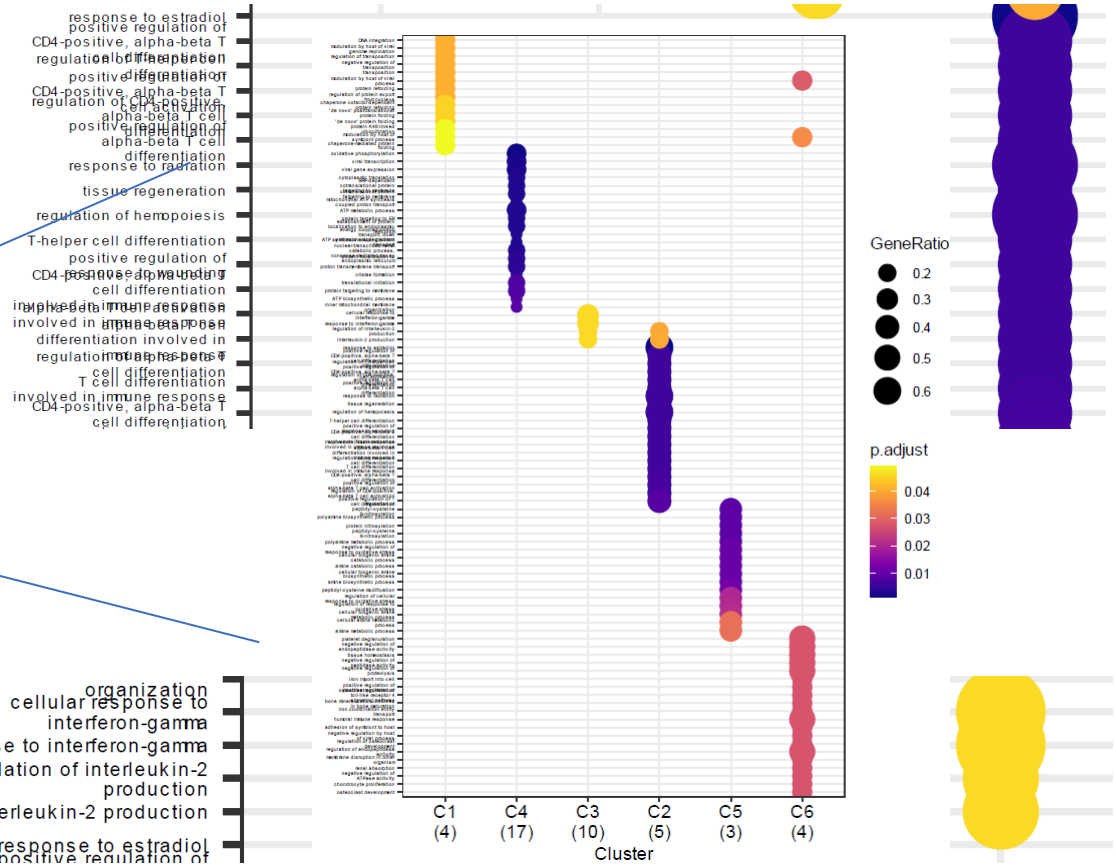
IL-2 suggests T cell infiltration

EpCAM High EpCAM Low





EpCAM High PCA outliers lack EpCAM transcript yet were in EpCAM High FACS gate



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Summary - ResolveOME

- ✓Single-cell multiomic analysis combining **primary template-directed amplification with full transcript reverse transcription**
- ✓Unified workflow interrogating **DNA and mRNA from the same cell**
- ✓NGS library preparation kits included with ResolveOME kits

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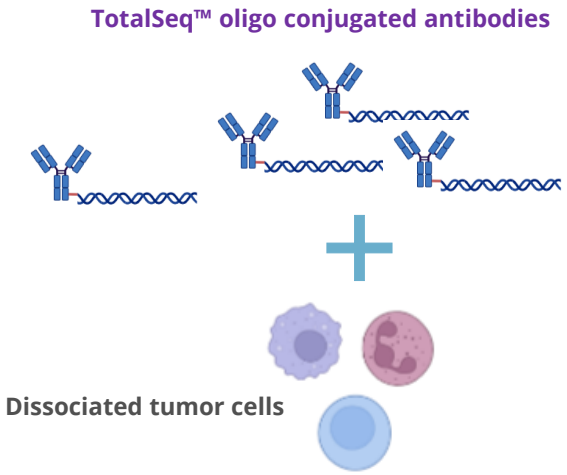
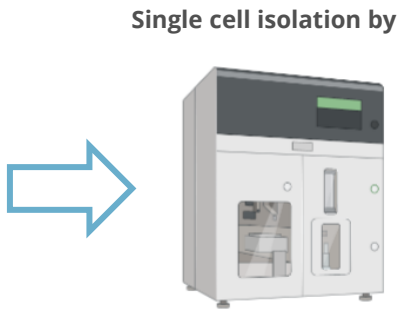
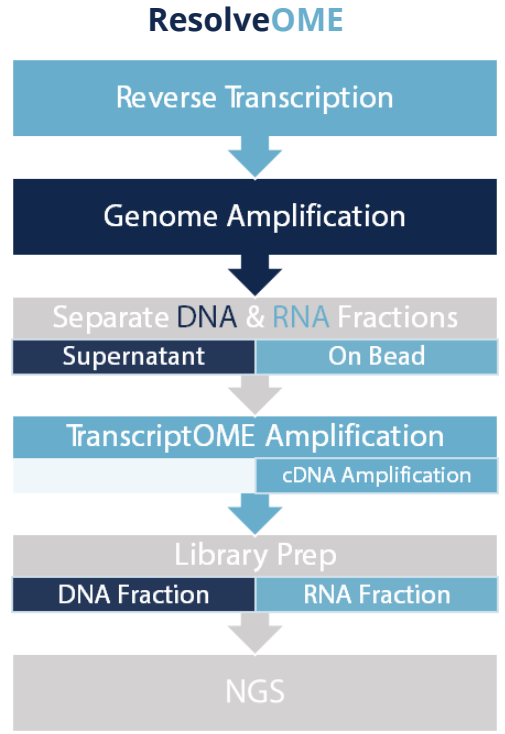
Cell Surface Marker Detection
DNA, RNA, and Extracellular Protein

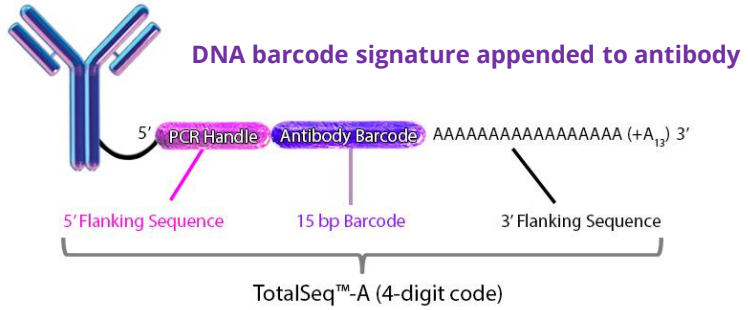
We can generate a next-generation sequencing (NGS) readout for antibody binding in **ResolveOME**!







BioLegend®
Enabling Legendary Discovery™



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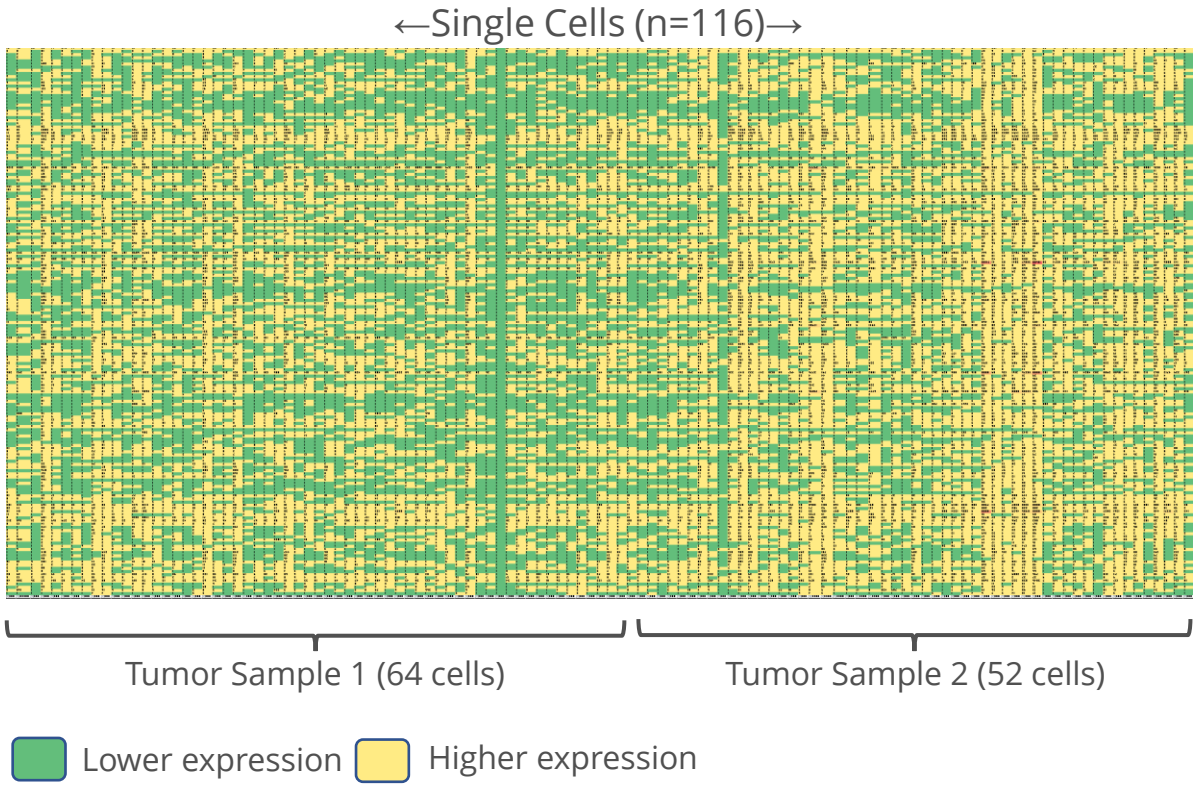
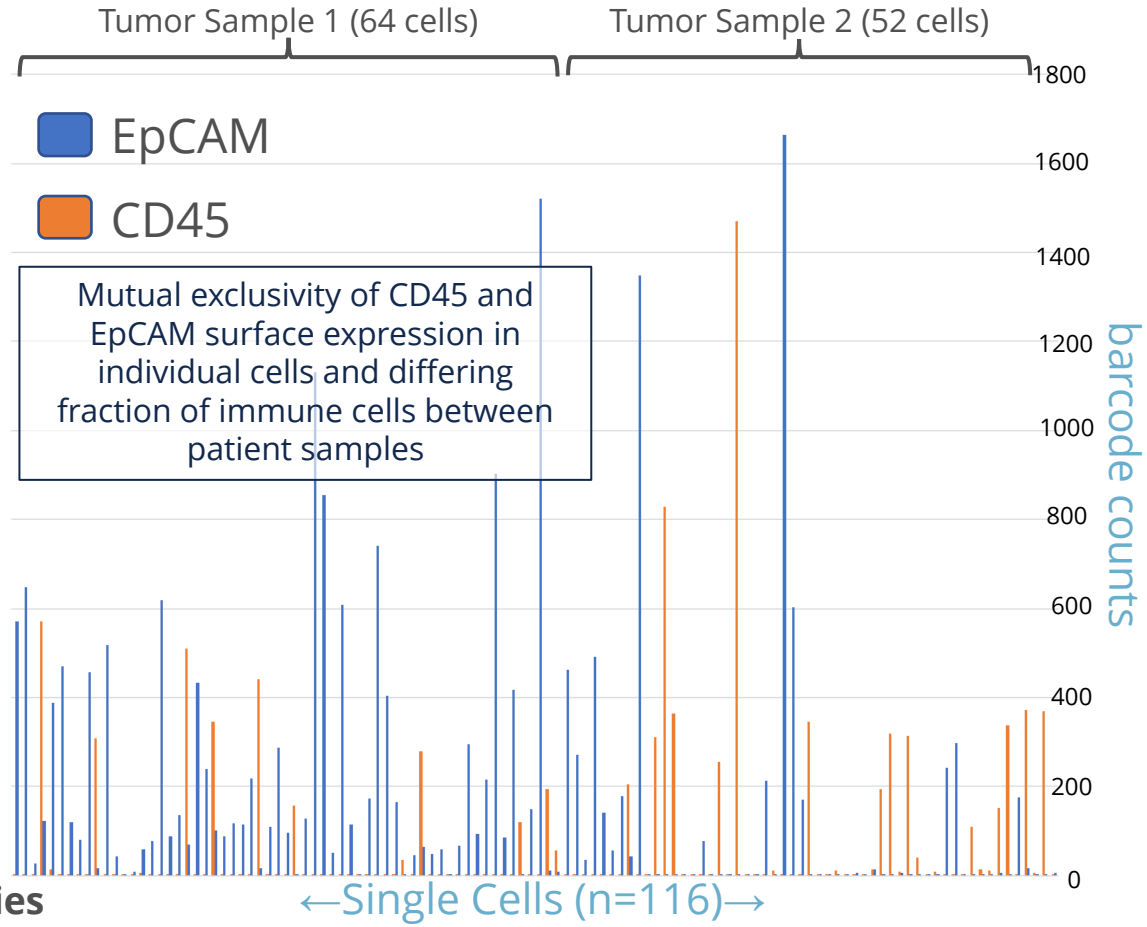
Exposing Single-Cell Surface Protein Profiles in DCIS Samples





154 BioLegend TotalSeq Universal Panel 1.0 oligo conjugated antibodies
+ 11 "epithelial" oligo conjugated antibodies
= 165 oligo conjugated antibodies

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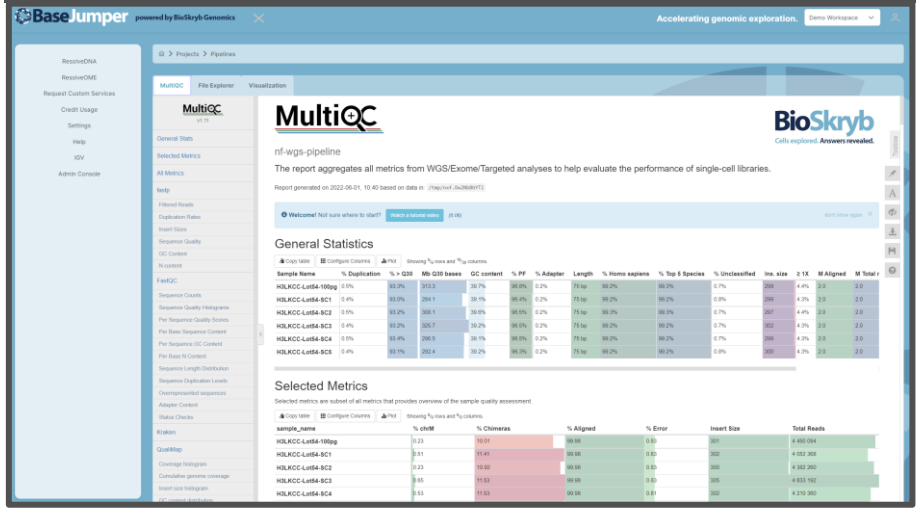
BaseJumper™ Multiomic Analysis Solution

BaseJumper is a scalable cloud-based solution that enables large dataset interpretation

- Single cell multiomics is redefining how complex tissues and illnesses are studied.
- Researchers can examine their own multiomic single cell data.

MultiQC Quality Reports



Simple, User Friendly Interface

Easily Accessible from Anywhere

Built in Step-by-Step Workflows

Robust Pipelines for QC and Multi-Omic Analyses

Secure Data and Project Analyses Storage

Fully Integrated and Interactive Visualizations

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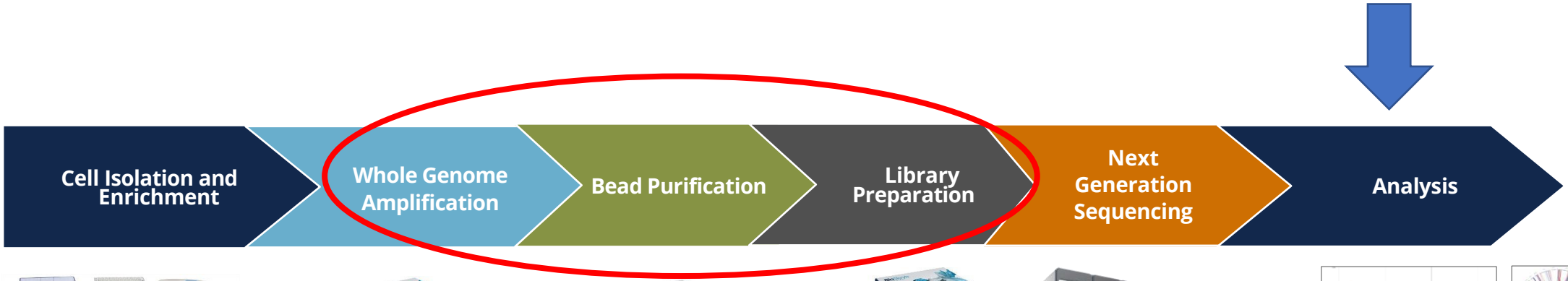
End-to-End Single-Cell Omics Workflow



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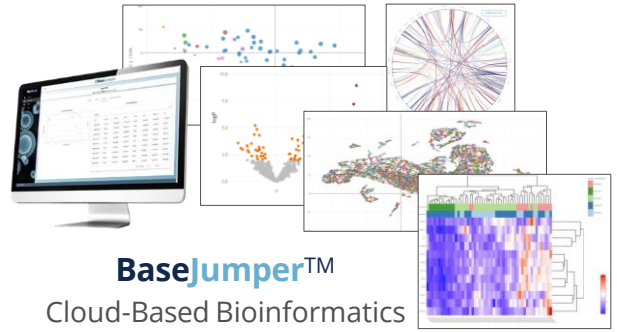

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xinkun.wang@northwestern.edu
Director, NUSeq Core Facility

John Bon – john.bon@BioSkryb.com
Christina Ulrich – Christina.Ulrich@BioSkryb.com
Andrew Rohs – Andrew.Rohs@BioSkryb.com

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