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

Whole Genome
Full Length Transcriptome
Same Single cell

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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
End-to-End Single-Cell Omics Workflow

Cell Isolation and Enrichment

Whole Genome Amplification

Bead Purification

Library Preparation

Next Generation Sequencing

Analysis

ResolveDNA®
Complete Starter Pack

ResolveOMÉ™
& ResolveDNA®
Whole Genome Amplification Kit

ResolveOMÉ™
& ResolveDNA®
Bead Purification Kit

ResolveOMÉ™
& ResolveDNA®
Library Preparation Kit

BaseJumper™
Cloud-Based Bioinformatics

Whole Genome or Whole Exome SEQ
The Technology Underpinning BioSkryb: Primary Template-Directed Amplification (PTA)

More Uniform Genome Coverage

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

Superior Allelic Balance

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

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

Sensitivity are based on positions that are 25X coverage for ResolveDNA, values for alternative methods taken from Gonzalez, et al. PNAS. CV: Coefficient of Variation; SNV: Single Nucleotide Variant

ResolveDNA outperforms other common methods with respect to data quality and variant calling metrics.
Indels in gene-regulatory elements have a considerable effect on genome integrity in human neurons

- 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
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
Characteristics of ResolveDNA in Single Nuclei from PFCs

Copy number variation

Amplification uniformity

Allelic balance

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

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

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
PTA Outclasses MDA-based Approaches in somatic SNV & InDel

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

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”

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

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

Luquette et al., Nature Genetics 2022
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.
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.
ResolveOME: A Single Cell’s Most Complete Genome and Transcriptome

<table>
<thead>
<tr>
<th>DNA Performance Characteristics</th>
<th>Observed Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>99.5%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.1%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.2%</td>
</tr>
<tr>
<td>Precision</td>
<td>99.5%</td>
</tr>
<tr>
<td>Allelic Balance</td>
<td>98.4%</td>
</tr>
<tr>
<td>Genome Coverage</td>
<td>97.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RNA Performance Characteristics</th>
<th>Observed Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes Detected</td>
<td>4,546 Genes*</td>
</tr>
<tr>
<td>Reportable Range</td>
<td>6057</td>
</tr>
<tr>
<td>Average Concordance</td>
<td>0.91</td>
</tr>
<tr>
<td>Reproducibility (CV)</td>
<td>43.3%</td>
</tr>
</tbody>
</table>

*NA12878/HG001 cells
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.
ResolveOME High Quality Transcript and Genome Data

- 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

More information on our bioRxiv paper Zawistowski et al., 2022
Ductal Carcinoma in Situ (DCIS) Collaboration – Customer study

- Surgical Resection of Tumor and Tumor Normal
- Cell Dissociation
- Cryo Preservation
- Facs Enrichment
- Resolve Chem. SC-AMP
- Library Prep
- Low depth Sequencing
- High depth Sequencing
- BaseJumper Analysis

BioSkryb's ResolveDNA pipeline provides an end-to-end solution, supplying ready to use kits and protocols for FACS, WGA, library preparation and bioinformatic analysis using BaseJumper.
CNV analysis: Patient TME-19-016

Normal breast single cells

Tumor single cells

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
Copy Number Insights Are Missed by Bulk Sequencing

Bulk captures some predominant alterations seen in single cells...

Diversity of cell types in a tumor sample mutes rarer alterations

...but misses other discrete alterations in EpCAM enriched 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
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
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
We can generate a next-generation sequencing (NGS) readout for antibody binding in ResolveOME!

TotalSeq™ oligo conjugated antibodies

Dissociated tumor cells

Single cell isolation by FACS

ResolveOME

Reverse Transcription

Genome Amplification

Separate DNA & RNA Fractions

Supernatant

On Bead

TranscriptOME Amplification

cDNA Amplification

Library Prep

DNA Fraction

RNA Fraction

NGS

BioLegend®
Enabling Legendary Discovery™

DNA barcode signature appended to antibody

5' Flanking Sequence

15 bp Barcode

3' Flanking Sequence

TotalSeq™ A (4-digit code)
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

Mutual exclusivity of CD45 and EpCAM surface expression in individual cells and differing fraction of immune cells between patient samples
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.
End-to-End Single-Cell Omics Workflow

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