



Single Cell Transcriptomics

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- Cellular Research, Inc.
- *Prepared for the 2015 AACR Conference:
Emerging Clinical & Laboratory Diagnostics: Pushing the Envelope*

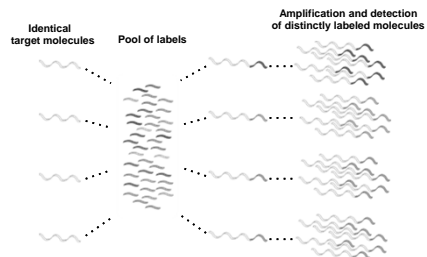


Learning Objectives

- Molecular indexing: technology background
 - Labelling approach for high accuracy and sensitivity
- Extension of molecular indexing to single cell sequencing outputs
 - Precise™ with application examples
- Massively parallel single cell transcriptomic data from microwell arrays: the Resolve™ system



Molecular Indexing



Fu et al, PNAS 2011
Fodor et al, United States Patent 8,835,358



Molecular Indexing workflow – 1

WHOLE TRANSCRIPTOME ENCODING

During the Molecular Indexing reverse transcription process, each and every mRNA molecule is randomly encoded with a unique Molecular Index, thereby producing an indexed cDNA library. In this library, every cDNA molecule is permanently encoded and can be repeatedly amplified and quantified with no loss or bias.

HOW IT WORKS



Molecular Indexing workflow – 2

Co-amplifying transcripts bound to Molecular Indexes results in absolute quantification regardless of amplification bias, because each index identifies a unique starting molecule.

HOW IT WORKS



Molecular Indexing workflow – 3

DETECTION

All molecules sharing the same Molecular Index are counted as a single starting copy number, minimizing inevitable variations in amplification efficiency, detection method or workflow variance from affecting the results.

HOW IT WORKS



Applications of Molecular Indexing

- High Throughput Cell based assay for compound screening
- Sequencing of mRNA from Exosomes and FFPE samples
- Target validation of genes after whole transcriptome sequencing
- High throughput single cell analysis



Recent Articles Using Molecular Indexing

Science Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types
 Deep Adhikari^{1,2}, Eyalit Avigdal^{1,2}, Rupa Anand^{1,2}, Rama Arora^{1,2}, Anand Bhat^{1,2}, Chao Chang^{1,2}, Alexander Chikina^{1,2}, Stefan Diehl^{1,2}, Anshu Dutt^{1,2}, Wei Han^{1,2}

nature Quantitative single-cell RNA-seq with unique molecular identifiers
 Khalid Elmar¹, Anshu Dutt¹, Shantanu Das¹, Gauri Va. Mahajan¹, Prasad Zuber¹, Maria Krupar¹, Peter Linschoten¹, & Ronen Lancetman¹

PNAS Molecular indexing enables quantitative targeted RNA sequencing and reveals poor efficiencies in standard library preparations
 Shrawan A. Patel¹, Shrawan A. Patel¹, Anshu Dutt¹, Michael W. Strother¹, Ronald W. Davis¹, Shrawan A. Patel¹, and Shrawan A. Patel¹

nature biotechnology Linking T-cell receptor sequence to functional phenotype at the single-cell level
 Anand Bhat¹, Jacob Chavakis¹, Leo Hoesly¹, & Mark M Davis^{1,2}

Science Combinatorial labeling of single cells for gene expression cytometry
 Erik Wigmore¹, Zhen Wang¹, Yuxia Zhou¹, Chad Leland¹, Dan Chishti¹, Robert Binkley¹, Scott Taylor¹, Peter Hensler¹, Tom Freeman¹, & Steve Liaw¹

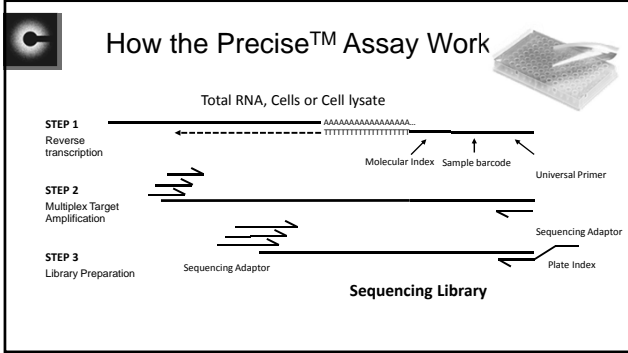


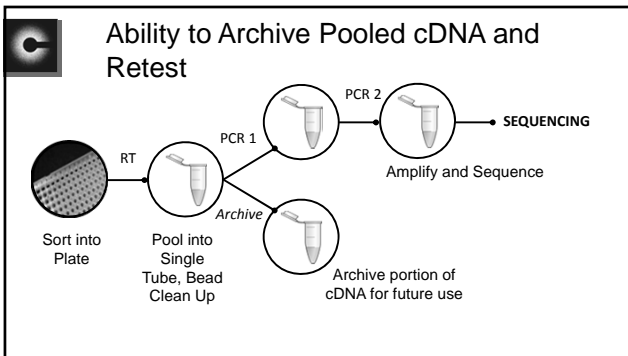
96-Reaction Molecular Indexing: Precise™

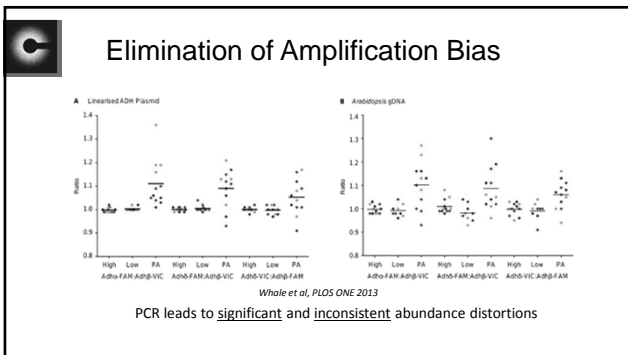
- Microtiter plate format: use downstream of cell sorting
- Unique identifier sequence in each well



- High Throughput
 - Single tube workflow after pooling 96/384 samples
 - Multiplex up to twelve plates per sequencing run
- Accurate Digital Data
 - Molecular indexing reduces amplification bias and enables direct quantitation
 - 3' anchoring enables highly multiplexed PCR sampling

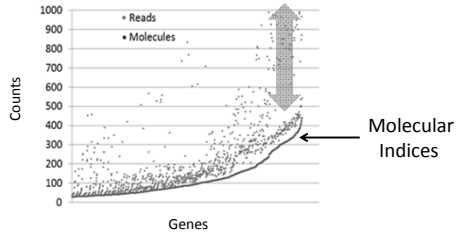








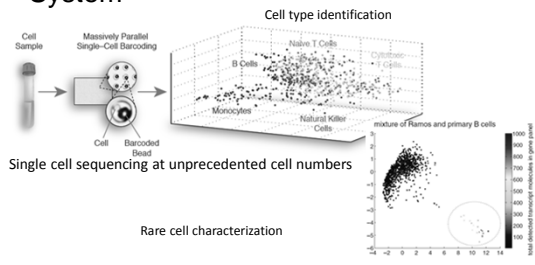
Molecular Indexing Corrects for PCR Bias



Fu, et al. Analytical Chemistry, 2014.



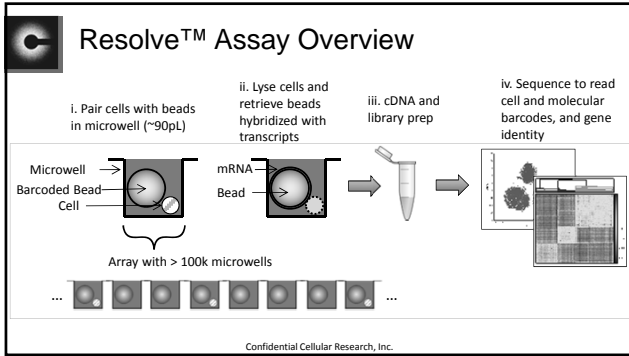
Massively Parallel Single-Cell Transcriptomics: the Resolve™ System

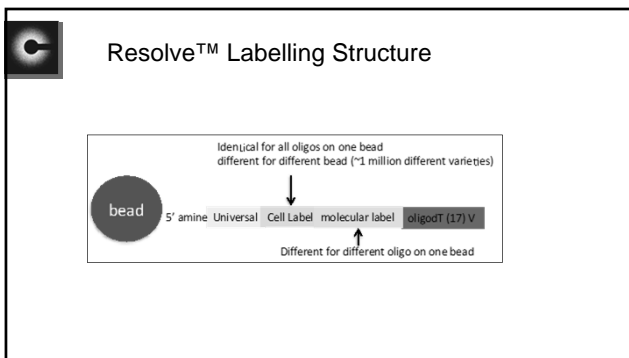




Have Your Cake and Eat It: Massive Cell Numbers with Single-Cell Resolution

- Benefits of single cell data
 - Sample heterogeneity
 - Rare cell characterization
- Robustly traceable transcript counts
- Single tube workflows





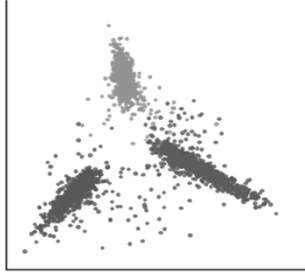
Cell statistics during assay feed into final sequencing data analysis

- Instrument for automated imaging and analysis
- Assay performance statistics, e.g.:
 - # cells
 - pairing efficiency with beads
- Additional info beyond
 - Viability (2-channel fluorescence)
 - Cell size and similar attributes
- Evaluate the experiment before committing to sequencing



Example Resolve™ Sequencing Data

- Experiment parameters:
 - 3 cell lines
 - Multi-gene panel
- 3000 cells
- PCA analysis:





Support

- Cellular Research, Inc.
- SBIR Grant 1R44HG008323-01
- Thanks also to the AACC organizers and session chairs
